The Properties and Effects of Dust from Unpaved Roads on Vegetation and Microbes in the Karoo

by

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Summary

There is minimal information on how dust from unpaved roads affects plant communities in the semi-arid Nama and Succulent-Karoo. One site per biome were used for conducting research, including the Square Kilometre Array (SKA), falling within the Nama Karoo biome and Wolwekraal Nature Reserve, falling within the Succulent Karoo biome. Each site was divided into four distance categories (D1 = 0–20 m; D2 = 20–100 m; D3 = 100–400 m; and D4 = 400–1000 m) from their respective unpaved roads. Within each distance category Modified Wilson and Cooke (MWAC) samplers were placed, on the soil surface and 1,3 m above the surface using steel rods and soil samples (including the road) were taken to a depth between 5 to 10 cm. After two months of dust sampling, samplers were collected to analyze the physical characteristics of dust (load and particle size); and microbial characteristics (fungal and bacterial composition and species richness) were determined in both the samplers and soil samples. Leaves from two shrubs (*Pteronia glauca* and *Rhigozum trichotomum*) in SKA and one shrub (*Pteronia pallens*) and a succulent (*Ruschia spinosa*) in Wolwekraal were sampled in each distance category. The leaves were analyzed for their leaf specific area (SLA), leaf stable carbon and nitrogen isotopes and metal ion deposits (Cu, Zn Cr, Pb and Ni). Results indicated unique patterns between sites. Mean dust loads were highest closest to the road (0-20 m) at both sites, and decreasing mean dust loads and particle size with increasing distance from the road were evident at SKA whereas mean dust loads and particle size displayed an inconsistent pattern along the distance gradient at Wolwekraal. Among the five metals Cr, Pb and Ni are carcinogenic and only these three metals had the highest concentration in the distance category closest to the road (0-20 m). Despite high dust loads and metal concentrations in the distance closest to the road it did not seem to significantly impact, with the exception of $\delta^{15}N$ in *P. glauca* at SKA, the physiological indicators included ($\delta^{15}N$, $\delta^{13}C$, and SLA), which suggests only minor water and nitrogen-use impacts on plants, due to dust deposition. Dust transported from the road had low microbial species richness and influenced the samplers in the 0-20 m zone and associated soil samples by diminishing their microbial richness and diversity at SKA. Understanding the effects of dust pollution on plants and soil microorganisms would aid conservationists to better understand the nature of the environmental impacts facing arid and dust-prone areas. Based on the findings of the study it is recommended that implementation of dust mitigation techniques is applied in order to minimize dust generation from unpaved roads.

Opsomming

Daar is minimale informasie wat focus op hoe stof van ongeplaveide paaie die plant gemeenskap in die semi-droë Nama en Sukkulent-Karoo beinvloed. Navoring het plassgevind binne een plek per bioom, insluitend die Square Kilometre Array (SKA), wat binne die Nama-Karoo val en die Wolwekraal Natuurreservaat, wat binne die Sukkulent-Karoo val. Vanaf die respektiewe ongeplaveide paaie was elke plek in vier afstand kategorieë verdeel (D1 = 0–20 m; D2 = 20–100 m; D3 = 100–400 m; and D4 = 400–1000 m). Binne elke afstand kategorieë was Modified Wilson and Cooke (MWAC) opvangers gepelaas, op die grondoppervlakte en 1,3 m bo die grondoppervlakte met behulp van staalstawe en grondmonsters was geneem tot 'n diepte tussen 5 en 10 cm. Na twee maande van stofopneming, was die stofmonsers versamel om te analiseer wat die fisiese eienskappe van stof (lading en partikelgrootte) was; en mikrobiese eienskappe (fungus- en bakteriële samestelling en spesierykheid) was vir beide stofmonsters and grondmonsters bepaal. Blare van twee struikplante (*Pteronia glauca* en *Rhigozum trichotomum*) by SKA en een struikplant (*Pteronia pallens*) en een vetplant (*Ruschia spinosa*) in Wolwekraal was in elke afstand kategorieë versamel. Die blare was geanaliseer vir hul spesifieke blaaroppervlakte, stabiele koolstof- en stikstofisotope en metaalioonafsettings (Cu, Zn Cr, Pb, and Ni). Gemiddelde stoflading was hoogste naaste aan die pad (0-20 m) by beide plekke, en n afneming van stoflading en partikelgrootte met toenemende afstand vanaf die pad was duidelik by SKA, maar gemiddelde stoflading en partikelgrootte het n inkonsekwente patroon met die afstandgradiënt by Wolwekraal vertoon. Van die vyf metale dra Cr, Pb en Ni karsinogeniese eienskappe en hierdie metale was die hoogste in konsentrasie by die afstand naaste aan die pad (0-20 m), en het nie n aansienlike impak, met die uitsondering van ^{15}N in *P. glauca* by SKA, op die fisiologiese aanwysers (δ^{15}N, δ^{13}C, and SLA) van die plante gehad nie, wat aandui dat daar slegs 'n geringe invloed op die gebruik van water en stikstof op plante was, as gevolg van stofneerlegging. Stof wat vanaf die pad vervoer was het lae mikrobiese spesierykheid gehad en dit het stofmonsters in die 0-20 m afstand en gepaardgaande grondmonsters beïnvloed deur hul mikrobiese spesierykheid en diversiteit by SKA te verminder. Om die gevolge van stofbesoedeling op plante en grondmikroörganismes te verstaan, sal natuurbewaarders help om die aard van die omgewingsimpakte wat droë en stofgevoelige gebiede in die gesig staar, beter te verstaan Op grond van die bevindinge van die studie word dit aanbeveel dat die toepassing van stofversagtingstegnieke toegepas word om die opwekking van stof vanaf ongeplaveide paaie tot 'n minimum te beperk.

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1.1. General introduction

Dust is generated by activities relating to agriculture, cement factories, mining, and power plants, but also increasingly by road transport as traffic volumes increase (Chaturvedi, 2013). Extensive research on dust across the globe has been undertaken, however, most research has been carried out in developed countries such as the USA and Australia and only few studies in middle-income countries like South Africa. Although a large body of research on dust exists, only a few studies are relevant to gravel roads and even fewer provide information on the quantitative impacts of dust (Greening, 2011). In the USA about 2.5 million km of unpaved roads have been recorded, which is estimated to be just below half of the entire US highway system (Barnes, 2014). Studies have shown that unpaved roads in South Africa generate more than three million tonnes of dust a year (Jones, 2000). There is approximately 500 000 km of unpaved roads in South Africa according to Jones (2000), however, Greening (2011) suggests 600 000 km of unpaved roads. The effect of dust on the physiological and structural properties of plants in South Africa is poorly understood. Assessing and understanding how dust generated from gravel roads and other infrastructure affects vegetation can be particularly important for biodiversity conservation (Lewis et al. 2017). For example, road dust threatens vegetation communities by inducing changes to respiration, transpiration, and photosynthesis, especially if toxic pollutants penetrate stomata or deposit on leaves (Kumar, 2014).

Dust is a significant factor that remains overlooked in ecological studies, especially at the local or landscape scales, despite the fact that dust can induce morphological or biochemical changes that in turn puts pressure on the ability of plants to react and adapt to abrupt changes in the environment (Sarma et al. 2017). Mineral dust is mainly composed of coarse particles and often associated with mining activities, but can also arise from road traffic (Chaston and Doley, 2006). By means of air currents, the dust can cling to the upper and lower portion of the leaves when passing over plants. What happens next depends entirely on the size and character of the particles, the surface area on which the particles are deposited and the velocity of the wind (Sett, 2017). Dust accumulation on plant species varies due to the different characteristics of the leaves, the canopy, and the height of roadside plants (Sarma et al. 2017). Climatic conditions i.e., rainfall is also an important factor that plays a role in dust accumulation and removal from the plant leaves (Rahul and Jain, 2014).

Dust load, particle size, and mineral composition are all important characteristics when considering the biochemical reactions in plants (Farmer, 1991). Deposited dust particles with diameters smaller than that of the leaf stomata can enter the cavity and come into direct contact with leaf parenchyma, whereas the larger particles can enter the leaf interior through dissolution caused by the release of carbonic acid of stomata itself or in water. Particle size is also important when considering the blockage of the plant's stomata, resulting in a decreased rate of CO_2 exchange (Sett, 2017). Generally, particle sizes smaller than 10 micrometres (called PM_{10} or particulate matter 10), disrupt the functions of the stomata. Clogged stomata reduce carbon assimilation and transpiration, which then negatively affects the overall plant physiological functioning (Ulrichs et al. 2008). The level of dust deposition is variable, and it has been shown that paved roads produce a lot less dust load per car than unpaved roads (Roberts et al. 1975). Thus, in theory, plants adjacent to paved roads should be significantly less impacted. A study on cucumber plants and kidney beans conducted by Chaston and Doley (2006) found that with increasing dust, leaf temperature increased while light penetration and photosynthetic rates were reduced. Dust particles contain various toxic metals and polycyclic hydrocarbons that inhibits the production of enzymes involved in chlorophyll construction resulting in an overall lower photosynthetic rate of the plant (Abu-Roman and Alzubi, 2015).

There are many gaps in the literature relating to the impacts of dust on vegetation. The ever-increasing concentration of industrial dust particles has received more attention in the last few decades because of the serious plant- and human-health impacts they can induce (Ulrichs et al. 2008). In particular, key components of dust, such as the load, chemical composition and particle size, have received more attention (Zia-Khan, 2015). In South Africa, few studies have evaluated the effects of dust on the physiological/biochemical performance of plants. Yet, a considerable amount of the literature reports negative effects of industrial dust on plant morphology, physiology, and biochemistry. That said, few have also investigated any positive consequences, such as fertilization.

Potentially, fungi and bacteria may also be transported from more intact and diverse areas to those previously disturbed, aiding recovery of microbial diversity in the disturbed areas. Soil and its biodiversity have always been disregarded as an important factor; however, in recent times more people recognize the value of soil micro- and macro-biota and their interactions in ecological processes (Wall and Moore, 1999). Currently there still exists a gap in understanding the relationships between ecosystem services, processes, and components of biodiversity, and how to utilize these relationships in dust management strategies (Groot et al. 2010).

In modern times humans have been very reliant on road transport, but it is a major source of dust as vehicles degrade the structure of the soil in unpaved roads, further making the roads and surrounding vegetation more vulnerable to erosion damage (Chaturvedi, 2013). Soil erosion is exacerbated by vehicle pressure, especially under dry climatic conditions when the shear stress dislodges particles more easily, which can be transported aerially by natural winds or generated by driving vehicles. Furthermore, when dislodged soils encounter surface water flow, gullies or rills form that may obstruct travelling on roads (Ngezahayo, 2019). Thus, the rate of dust generation is believed to be a factor of the frequency of vehicles and poor road infrastructure (Sarma et al. 2017). In view of the above, I set out to explore the potential effects that dust might have on vegetation and distribution of microbes, to help fill the current gaps in the literature on dust generation and biodiversity conservation in an era of rapid environmental change. The rest of Chapter 1 is therefore devoted to a literature review on the topic, and subsequently followed by the research aims and objectives of this study.

1.2. Literature review

1.2.1. General impacts of dust aerosols

Very few greenhouse or field experiments are available to help understand the potential impacts of airborne particulate matter on vegetation (Rai, 2016). This lack of information is due in part because people only recently became more aware of the impacts of particulate matter on ecosystems (Cwiklak et al. 2007). There is thus a need for more studies on airborne particulate matter emissions (PMEs), as those who had studied this subject cautioned that it can negatively affect the fitness of sensitive species and potentially disrupt plant diversity, growth and development, ultimately altering the structure and dynamics of the exposed vegetative community (Pereira et al. 2009).

Atmospheric aerosols contain fine dust which is called particulate matter (PM). The PM with a diameter of <2.5 µm ($PM_{2.5}$) or <10 µm (PM_{10}) has been widely studied in recent years, because if present in high concentrations within particular ecological niches, it can pose a serious health threat to all living organisms, including humans (Prajapati and Tripathi, 2008). A biome can be affected at different organizational levels by PMEs. PMEs directly affect the plants by means of abrasion, or indirectly by interfering with its physiological functioning, e.g., stomatal blockage leading to changes in temperature and gas conductance (Suárez-Esteban et al. 2014). Particulate matter at the individual level can affect processes such as reproduction, mineral nutrition, *in situ* temperature and photosynthesis. This can happen due to physical abrasion and obstruction of the stomatal pores or by chemical effects such as changes in pH and oxidation levels. But even more severe are impacts at the population- and community-level, where particulate matter can alter energy flows and nutrient cycles (Pereira et al. 2009).

Because plants are sessile, they cannot move from harmful contaminants, making them important bioindicators in many ecosystems. Air pollution can be considered a multi-stressor agent as it can lead to changes in the physiological responses of plants, such as membrane damage and changes in certain enzyme activities, which is essentially why plants are considered good pollution load indicators (Majumder et al. 2015). Though there has been little work done on trying to find out how particular plants respond to these changes in particulate matter pollution, over the last three decades there has been a greater focus placed on this topic—mainly due to the negative biological impacts that result in lower economic returns (Cwiklak et al. 2007). Many studies indicate similar trends of adverse biochemical and physiological impacts of particulate matter on plants.

The degree to which a vegetative community is disrupted depends on the combination of the physicochemical properties of dust (dust load, particle size and chemical composition) (Farmer, 1991), local environmental conditions (wind velocity, rainfall, humidity and temperature) and the characteristics of the plants on which dust is deposited (canopy height, petiole length and various leaf traits, e.g., leaf geometry and the presence of cuticles and hairs) (Gupta et al. 2016). Due to rising anthropogenic polluting activities, such as mining, road dust generation and industrial processes, as well as climate change many ecosystems close to such sources have been threatened (Sett, 2017). Thus, it is essential to assess and comprehend how dust generated from these sources affects vegetation structure and dynamics in order to mitigate effects and improve biodiversity conservation planning and management (Lewis et al. 2017).

1.2.2. Dust characteristics

1.2.2.1. Dust loads

Throughout many countries and in different biomes, studies were conducted to determine the total amount of dust loads. Dust load was determined either by means of using a particular dust sampler to capture dust from anthropogenic sources or measured on a pre-set amount of leaves from various species. The sampling period and sampling season are important factors that play a role in the penultimate amount of dust captured by samplers or plant leaves. Table 1.1 shows the variety of dust sampling methods used in the literature and give examples of the dust loads captured.

Table 1.1. Studies on dust loads captured, including the county and its biome, the dust sampler/plants species used, and the sampling period.

Country	Biome	Dust sampler/plant species	Dust load	Sampling season	Sampling period	Reference
Western Nepal	Tropical (dominated by south west monsoon)	*Delonix regia*	0.093 ± 0.1016 g (highly polluted area) 0.016 ± 0.008 g (low dust pollution area)	Summer	Not available	Nepali and Gyawali (2001)
India	Sub-tropical (valley) and temperate (hilltop)	*Toona ciliata*	0.063 g m^2 (rainy season) 0.094 g m^2 (dry season)	Mainly winter (dry), summer and rainy August	10 months	Kaler et al. (2016)
India	Tropical	*Calotropis procera*	2.02 mg cm^2 (within 500 m of factory)	Not available	Leaf samples collected between 8 and 10 AM	Swain et al. (2016)
China	Mongolian Grassland	Collection cup	0.26 g m^2 d^{-1} (upwind) 0.59 g m^2 d^{-1} (downwind)—500 m away	Spring and Summer	Apr–Sep of 2013	Zhan-Yi et al. (2016)
China	Tropical	*Quercus variabilis* *Platanus occidentalis* *Cephalotaxus sinensis* *Broussonetia papyrifera*	127.0–160.9 µg cm^2	Spring	April 2014 (start of growing season)	Mo et al. (2015)

Country	Biome	Dust sampler/plant species	Dust load	Sampling season	Sampling period	Reference
America	Texas chemical plants	Cyclone samplers Cascade impactor samplers	3.62 mg m^{-3} 2.64 mg m^{-3} (Based on British Medical Research Council criteria)	Summer	July 21–July 29	Groves et al. (1994)
India	Monsoonal	*Ficus bengalensis*	0.184 mg cm^{-2} (dry season) 0.013 mg cm^{-2} (rainy season) (Graph approximation)	Winter (dry), summer and rainy season	November 2011–February 2012	Rai and Panda (2014)
India	Monsoonal	*Terminalia arjuna* *Morus alba*	57 ± 3, 120 ± 5 and 117 ± 7 mg m^{-2} d^{-1} (Monsoonal, winter, summer) 182 ± 5, 349 ± 7 and 285 ± 14 mg m^{-2} d^{-1} (Monsoonal, winter, summer)	Monsoon Winter Summer	2012–2014	Gupta et al. (2016)
Arctic Alaska	Tundra and Alaskan Taiga	890-cm^2 pans	24 kg ha^{-1} d^{-1} (0-10 m) 0.6–0.02 kg ha^{-1} d-[1] (30–1000 m)	Summer	1977–1978	Walker and Everett (1987)

Country	Biome	Dust sampler/plant species	Dust load	Sampling season	Sampling period	Reference
USA	Mojave Desert	Marble Pans	0.04–0.17 g m^{-2} d^{-1}	All seasons	May 2004–July 2004	Wijayratne et al. (2009)
USA	A wetland landscape and the North-western Glaciated Plains	Bucket	647 g m^{-2} (high impact site—10 m) 197 g m^{-2} (low impact site—10 m) 171 g m^{-2} (high impact site—80 m) 132 g m^{-2} (low impact site—80 m)	Spring Summer Fall	April–October (214 days)	Creuzer (2016)

There are many factors that can potentially affect emission rates, which makes quantifying real-world dust emissions quite problematic in practice (Pirjola et al. 2010). From a physical viewpoint, there is wind dynamism. Particle movements are usually initiated by the combined effect of atmospheric turbulence and direct wind shear stress (Lancaster, 2009). Three modes of sediment transport by wind are recognized: saltation, suspension and surface creeping. The transport mode depends on the ratios between turbulence intensity, wind shear stress and dust particle size (Lancaster, 2009). Thus, if we take dust from unpaved roads as an example, the amount of dust on leaves can be determined from the expected road dust density generation due to traffic, but should preferably also account for weather or prevailing climate conditions (Rahul and Jain, 2014).

Road surfaces degrade overtime via wearing of granules, creating PM_{10}, which is especially in the case of sand containing more PM_{10} than other soil textures. However, PM_{10} emissions would also depend on the type of vehicle tire in question, with studded tires shown to increase PM_{10} emissions because they tend to increase road surface wear particularly when road conditions are dry (Pirjola et al. 2010). Vehicle speed also directly affects emissions. Kuhns et al. (2008), using TRACKER (Testing Re-Entrained Kinetic Emissions), a method used to measure a road's potential to emit dust, found that vehicle speed is directly proportional to dust emission rates on unpaved roads (Kuhns et al. 2008).

Zhan-Yi et al. (2016) sprayed two kinds of coal dust, namely granite powder and coal powder, onto five different plant species. They found that certain root morphological parameters gradually decreased as more dust was added to the plants daily. However, initially the root surface area, length and volume of the plants from the two coal dust treatments were larger than that of the control, but as time passed and dust load on the plants increased, root surface area, length and volume of the least resistant plants became significantly smaller than those in the control (Zhan-Yi et al. 2016). Similarly, Prajapati and Tripathi (2008) investigated chlorophyll pigment concentration in dust-loaded plants. The authors found that, in winter, when dust loads were highest, total chlorophyll were the lowest for the dust-loaded plants. Walker and Everett (1987) recorded a 10-cm thick dust blanket on soils 0–10 m adjacent from heavily travelled roads, which resulted in the burial of very low vegetation and the complete elimination of mosses. Within 30–100 m of the road, a decrease in soil lichens, particularly species of *Peltigera*, *Cladina* and *Stereocaulo*, were found. Yet, not all dust effects near roads are negative. For example, Li et al. (2014) found that plant diversity within 0–20 m from an earth and asphalt road was high, and so too was the number of halophytes and non-indigenous plants that occurred there. This was because the roads acted as a dispersal corridor for plant propagules, where animal movement was less restricted, and water runoff and wind could transport seeds (Li et al. 2014).

1.2.2.2. Dust particle size

The definition of dust given by the International Standardization Organization (ISO) is "small but solid particles that are usually smaller than 75 µm that can settle down under their own weight and has the ability to remain suspended in the air for some time (ISO 4225, ISO, 1995)." However, this definition is a simplification as the term "particle diameter" refers to its geometric measurement and does not in any terms explain its aerial behaviour (WHO, 1999). Generally, particles with a diameter of >50 µm do not remain airborne for too long; however, depending on environmental conditions, even particles >100 µm can soar through the air (WHO, 1999).

The quantity and quality of dust captured by plants depend on the plant functional type (i.e., density and dimensions of foliage elements) and particle size (Rahul and Jain, 2014). Using different plant functional types and various plant species, Mo et al. (2015) found discrete differences in both the quality (particle size differences) and quantity of dust on the leaves of the different species. This was due to the variations in the microstructure of the leaf surfaces (Mo et al. 2015). Plant leaf folds are important as this allows a plant to pick up different size particles more efficiently. In the folds, there was a mixture of many coarse particles with ultra-fine and fine particles present. Thus, plants with wrinkled leaves capture larger amounts of PM and a wider variety of particle size classes than plants with smoother leaves (Zanhong and Jibiao, 2006).

Although Mo et al. (2015) found no significant difference in the distribution of particle size between the adaxial and abaxial sides of the leaves, Ulrichs et al. (2008) found that the blockage of particulate matter happens to have a greater effect on the adaxial surface of the leaf opposed to the abaxial surface, however, the magnitude of the effect on either side depend on the particle size. Smaller dust particles cause a greater reduction in photosynthesis, presumably since a greater shading effect results from the more closely packed, smaller dust particles that prevent the leaves from obtaining the optimal light quality (Hirano et al. 1990).

1.2.2.3. Dust chemistry

Plants can be heavily affected by dust load and particle size, but the importance of dust chemistry is often ignored. The mineral composition of dust can vary depending on the particular soil source. Each particulate matter type would thus have its own individual characteristics, e.g., a different pH level, and consequently, make it challenging to broadly evaluate the impacts of particle pollution on vegetation. Farmer (1993) claimed that the effect of dust on plant leaves is affected more by its chemical composition than by the quantity of dust load. Leaf chlorophyll is highly sensitive to changes in pH (Farmer, 1993). When the pH of the leaf increases excessively, chlorophyll functioning may cease. Sometimes hydrated cement dust can liberate calcium hydroxide leading to increases in pH on the leaf surface (Farmer, 1993). There have been cases where alkalinity reached pH 12, a level of alkalinity that can hydrolyse lipid and wax components, denature proteins and infiltrate the cuticle ending in the plasmolyzation of the leaf.

Serious injuries on plant tissues may be inflicted when deposited dust has a pH ≥ 9 (Vardak et al. 1995). Several studies have indicated that when in the presence of free moisture, a cement kiln solution can reach pH levels between 10 and 12 due to the resulting hydroxyl ions. This resulted in severe leaf injury due to the degradation of the parenchyma and palisade cells (Darley, 2012). Depending on the specific location, many unpaved roads are responsible for producing dust containing calcium ions and various metals that can create an alkaline solution when coming into contact with moisture (Farmer, 1993). Alkaline dust with high MgO levels has been known to degrade the epicuticular waxes when deposited on the surfaces of *Picea abies* leaves (Bermadinger, 1998). The alkaline nature of dust can reduce a plant's ability to uptake certain minerals from the soil (Raajasubramanian et al. 2011). Dust of an acidic nature is mostly due to gaseous NO_x and SO_2 that produce acid radicals in the matrix of the leaf, adversely affecting the chlorophyll molecules (Turkand and Wirth, 1975).

The chemistry of dust is also important as the chemical composition plays a critical role in the colour of the dust. Chaston and Doley (2006) found distinct differences in leaf temperature when leaves were covered with light coloured flyash, grey overburden and dark coal dust. At 10 g m^{-2}, flyash increased the leaf temperature of *Metrosideros tomentosa* by 0.3 °C, overburden increased the leaf temperature of *Eucalyptus tereticornis* by 2 °C, and coal dust increased leaf temperature by 5 °C for *M. tomentosa*. There appears to be a clear increase in leaf temperature as the deposited dust gets darker (Chaston and Doley, 2006).

1.2.2.4. Dust microbiology

Microorganisms are highly diverse and play a key role in ecosystem maintenance and regulation. It is difficult to accurately estimate species richness for most of the taxa, but the efforts of researchers over many centuries found that microorganisms comprise the vast majority of species on earth. They occupy a wide variety of ecological niches; however, researchers do not completely understand their diversity patterns (Fierer and Lennon, 2011). Dust and its microbial communities could provide key information that relates to some of the unanswered questions in the field.

A pilot study conducted by Hanson et al. (2016) used indoor and outdoor dust samples to try and explain microbial community composition. They evaluated microbial communities in samples collected at school, home and from outdoor air samples by means of sequencing rRNA regions from fungi (18S and ITS) and bacteria (16S). Results showed that outdoor air samples were dominated by Gram-negative Proteobacteria whereas indoor dust samples were mainly comprised of Gram-positive bacteria. There was a great abundance of Basidiomycota fungi in outdoor dust samples and Ascomycota in indoor dust. In a transcontinental study of aeolian transported dust, Yamaguchi et al. (2014) demonstrated that phylogenetically diverse bacterial groups were transported 3000–5000 km downward from their source (China), which could impact ecosystems with indigenous microorganisms in the Pacific islands, Korea and Japan. Very few studies focussed on short distance transportation of microbial communities in the dust. Gardner et al. (2012) found highly diverse, i.e., up to 3000 sequences of bacteria, in wind-blown sediments that travelled from agricultural soils in Michigan. The transported bacteria included species from the phyla Proteobacteria, Firmicutes, Bacteroides, Acidobacteria, and Chloroflexi. This resulted in a great loss in productivity in agricultural soils since members of the phylum Proteobacteria execute important ecological roles in nutrient cycling that can improve soil fertility. However, even fewer studies evaluated the impacts of dust microbes on plants, which is an important aspect that future research should focus more on.

1.2.3. Impacts on plants

The few studies on dust impact on plants that were in the accessible literature generally reported a reduction in the photosynthetic capacity of the plant species that were assessed, with a few exceptions.

1.2.3.1. Physical impacts

The foliar part of plants is the main receptor of dust since they are continuously exposed to a polluting atmosphere (Kumar et al. 2014). When dust pressure becomes more severe it can be responsible for morphological changes within a plant (mostly in the leaf), which may induce adaptive evolution to survive within these difficult environmental conditions (Sarma et al. 2017). There is a strong correlation between changing environmental conditions and the functional and structural features of vegetation over short evolutionary time scales (Gostin, 2009).

Rai et al. (2010) reported the effects of dust on the growth- and micro-morphological features of ten annual plant species in a particular community. Remarkable differences over only two months were found. Reduction in plant growth, leaf area, size of epidermal cells and stomata were observed in all of the species. Many of the plants' cuticles were damaged, and because roadside plants already suffered from water loss, a well-developed cuticle may be crucial for plant fitness and survival (Rai et al. 2010). Gostin (2014) observed several leaf structural changes in five Fabaceae species near major roads. The foliar lamina of leaves, as well as the height of the palisade cells, decreased slightly in the presence of air pollution in all *Trifolium* species. All species had thicker leaf epidermal cell walls. Stomatal size also decreased in all of the species analysed. These size modifications are important responses in plants toward environmental stress to minimize absorption of pollutants from the atmosphere(Gostin, 2014). However, the extent of the morphologically negative effect depends on the leaf attributes of the plant. Chaturvedi et al. (2013) found that one out of four studied tree species, *Tectona grandis*, had a much greater decline in leaf area and other physiological parameters compared to the other three species. Upon closer inspection, the authors observed that *T. grandis* had a higher dust accumulation as it had a rough hairy surface and also a greater initial surface area compared to the other three species, the latter which had a markedly smoother leaf texture. Species with high dust-capturing abilities due to their leaf characteristics are thus ill-adapted to thrive in areas with high dust pollution (Chaturvedi et al. 2013).

1.2.3.2. Physiological impacts

The impacts of limestone dust produced from a limestone quarry on a succulent shrub (*Zygophyllum prismatocarpum)* within the Namib Desert had been investigated by van Heerden et al. (2006). There was a significant reduction in the plant's performance due to a decrease in electron transport and CO_2 assimilation and a reduction in chlorophyll content. However, the plants recovered as soon as limestone extraction at the quarry was terminated and rainfall removed most of the dust from leaves. Within South Africa the largest coal exporting port is on the northern Kwazulu-Natal coast, in Richards Bay. One of the dominant species within the site is *Avicennia marina*. Naidoo and Chirkoot (2003) evaluated the photosynthetic performance of *A. marina* species since it accumulates coal dust on its leaves on a regular basis. They found that the carbon dioxide exchange of the upper and lower leaves was significantly reduced, by 17% down to 39%. This was due to a significantly lower photosystem quantum yield, resulting in an overall lower photosynthetic performance and growth rate of the plant.

There is a direct link between a reduction in plant growth and a reduction in chlorophyll. Chlorophyll pigments are very sensitive to air pollutants and when under stress they undergo several reactions that can induce physiological, biochemical and morphological changes in plants (Rai, 2016). There have been numerous workers that observed photosynthetic pigment degradation by air pollution (Armbrust, 1986; Prusty et al. 2005; Pareira et al. 2009). Gunamani et al. (1991) observed that when the dust becomes soluble within the cell sap of the leaves it produces more alkaline conditions, which is the main reason for the degradation of chlorophyll, leading to a reduction in photosynthetic activity. However, not all literature indicated a negative effect of dust pollution on plant physiological performance, because the result depends largely but not entirely on the plant species and the characteristics of the dust (i.e., the source).

Zhan-Yi et al. (2016) investigated the photosynthetic activity of five different plant species in the Mongolian grasslands. Coal dust reduced the net photosynthetic rate of three species but did not affect the photosynthesis of the other two species. They attributed this to a higher resistance against drought and toxic chemicals by the two species, which were supported by other findings (Zhao et al. 2009). Floating dust is a weather phenomenon that occurs annually in south of Xinjiang Uygur, north-western China during early spring (Xue et al. 2017). Another study, emphasizing the importance of dust type, found an increase in stomatal conductance and photosynthesis within *Populus euphratica*. The lower temperature caused by shading from floating dust increased the concentration of CO_2 and water vapour, important attributes that contribute to photosynthesis. The high humidity in the floating dust allowed the dissolution of potassium, phosphorus, nitrogen and sodium in aerosols, which “fertilized” the plant (Xue et al. 2017). Similar findings were supported by Wang et al. (2016) and Erel et al. (2015).

Ascorbic acid is a cellular oxidant that tend to increase with an increase in dust load. It helps protect the plant against oxidative damage and combats air pollution. Similarly, the amino acid proline aids in the plant’s defense and is a good stress indicator. Under great stress (air pollution) the plant produces more of these amino acids (ascorbic acid and proline) (Gupta et al. 2016). Increases in the antioxidant enzymes (defense mechanism) of *Arabidopsis thaliana* by cement dust provide further support (Abu-Romman and Alzubi, 2015).

1.2.3.3. Autecological impacts

Waser et al. (2017) observed the effects of dust generated by traffic from unpaved roads on the number of seeds and pollen produced by four wildflower species along a distance transect. Dust loads were highest in the 30 m range from the road verge, and dust particles varied greatly in terms of shape and size. Dust load had a negative relationship with pollen load; that is, where more road dust was deposited (closer to the road) more dust particles were found on the stigmas resulting in lower pollen loads. However, to the surprise of the researchers, the number of seeds per flower did not vary consistently with road proximity, meaning lower pollen loads did not necessarily result in a lower seed set (Waser et al. 2017).

Mandal et al. (2016) tested the relationship of dust generated by roads on fruit falling and pollination of litchi and mango at sites with low, medium and high road dust exposure. For both tree species, there was no correlation between successful pollination and road dust precipitation. The correlation between fruit falling and dust loads was weakly positive. Dust precipitation was not the main factor of fruit falling but rather nutritional and mechanical factors (Mandal et al. 2016). In contrast Naik et al. (2006) found that plants that grew in the vicinity of a stone quarry area had premature flower and fruit abscission. Dust deposited on plants resulted in an increase in leaf temperature and transpiration, which lead to lower water potentials in plants, the main factor in ethylene synthesis responsible for abscission. This was further enhanced by the shading effect created by dust (Naik et al. 2006).

Mutualistic relationships between mycorrhizal fungi, proteobacteria, actinomycetes and a specific plant can give that plant a competitive advantage over others within a community

(Wall and Moore, 1999). In the most common cases, the plant in this relationship is responsible for providing shelter and carbon whereas the symbiont increases the plant's accessibility to limiting nutrients, such as phosphorus and nitrogen (Wall and Moore, 1999). Very few studies have looked into whether anthropogenic-related dust production could enhance this mutualistic relationship or not, especially given that some dust pollution does indeed contain some of the limiting nutrients required by plants. However, such nutrient fertilization by dust would ultimately depend on the chemical composition of the specific dust type which is directly linked to its source (Ulrichs et al. 2008). Nonetheless, if this is true, dust pollution has the potential to shape and alter the particular state of an entire community, by increasing some plant species' competitive ability to that of other species (Levin, 1998). Alternatively, dust pollution could cause dieback and decline to others—all the more reason why more research on the effects of dust on species and communities is necessary for conservation ecology.

1.2.3.4. Traits that enable plants to mitigate dust pollution

Plants can capture dust from the air by means of three processes, namely deposition of aerosols and particles on the leaf surface, the slowing of air movement that allow dust fallout of particulates on the leeward side of vegetation and by the absorption of particles by leaves (Prajapati and Tripathi, 2008). The ability of plants to capture and retain dust depends on the range of their attributes, which includes phyllotaxy, outside geometry and leaf characteristics such as surface texture, petiole length, orientation, and size, as well as the presence or absence of leaf hairs, cuticle or waxes (Manisha et al. 2014). This in combination with anthropogenic actions and climatic conditions, such as air currents and their velocities, plays a pivotal role in the ultimate amount of dust that remains on plant leaves (Manisha et al. 2014).

The epidermal and cuticular traits of leaves can be used effectively as a bio-indicator of dust pollution as they respond to it in a quantitative and not a qualitative manner (Set, 2017). To prevent the particulate matter from entering the stomata, a plant under stress can produce more trichomes near the epidermal layer that act as a protective layer (Rangarajan et al. 1995). Pal et al. (2002) found that plants exposed to automobile exhaust can be highly adaptable to mitigate the adverse effects of pollutants. As plant trichome length increased, the wax layer got thicker and stomatal density changed significantly. Needle-leaved tree species have a much higher adsorptive capacity than broad-leaved tree species. This is because leaves of needle-leaved tree species have a rougher surface, a higher stomata number arrangement density and can secrete sticky grease (Zhang, 2015). Manisha et al. (2014) observed the combining effect of wind and leaf characteristics of different plant species and how this affected the amount of deposited dust on plants. Plants that had thick hairy leaves with short petioles had the highest dust loads. Plants with smoother leaves and thinner lamina had their deposited dust more easily removed by air movement, particularly when wind currents were strong.

Plants in winter may experience more negative physiological effects (e.g., a decrease in chloroplast amounts and photosynthesis) as opposed to the other seasons. Climatic conditions like high rainfall and wind speeds, if present during winters, could result in lower dust deposition amounts (Prajapati and Tripathi, 2000). In turn, increased dust pollution in low rainfall winter regions (e.g., summer rainfall areas) might therefore potentially increase winter plant stress.

Mangrove ecosystems in South Africa are continually being subjected to anthropogenic impacts, which provided a basis for Naidoo and Naidoo (2003) to determine the impacts of coal dust on leaf micromorphology and photosynthetic performance of four mangroves in Richards Bay. Although dust did not occlude stomata on any of the leaves, it did significantly reduce photosystem II quantum yield, electron transport rate and CO_2 exchange in two mangrove species. The other two non-affected mangroves, *Bruguiera gymnorrhiza* and *Rhizophora mucronata*, did not accumulate as much dust due to their leaves being covered in a smooth and thick cuticle (Naidoo and Naidoo, 2003).

1.2.4. Plant community impacts

Plant communities may change when there are individual populations that are sensitive to particulate matter stress. The particular response to stress will, however, depend on the microhabitat and surrounding available resources, growth stage and plant genotype (important plant characteristics) (Levin, 1998). In harsh conditions plants use their stored energy for maintenance rather than growth and reproduction. This means that less competitive plant species could have a temporary advantage over other species in its community, which can result in the return of succession to an earlier stage (Levin, 1998).

Road dust pollution can alter the structure in a plant community in various ways. Road dust over a period of two weeks caused cotton plants to lose a large amount of photosynthate, which was never fully recovered along with an increase in respiration. This change in plant physiology significantly reduced the dry weight of plants (Brandt and Rhoades, 1972). Dust deposited on the top leaf surface halted the amount of radiation the plants could use for photosynthesis by reflecting light. Thus, it is important to note that dust pollution favours the establishment of species more equipped and better adapted at the prevention or removal of pollutant accumulation (Brandt and Rhoades, 1972).

Zaharopoulou et al. (1993) found that when dust from limestone quarries was deposited on the roadside shrubs or trees, the pH of the bark became more alkaline which promoted the development of many lichen species. This stipulates the importance of the dust type in context, because the chemical composition of the dust is related to its pH. Murphy et al. (1999) compared abundances of epiphytic lichens at various distances away from a coal-burning station. Lichen abundances increased with distance away from the coal station. But this distance and lichen abundance relationship differed for some lichen growth forms, depending on the host tree species (Murphy et al. 1999).

Brandt and Rhoades (1972) determined the impact of dust accumulation on the composition and structure of a forest community at two sites (limestone polluted vs. control). The number of shrubs and seedlings was more in the control site, indicating more favourable conditions for reproduction. On average, dusty sites only had 7595 stems/ha whereas the control site had 11,305 stems/ha (Brandt and Rhoades, 1972). Differences in species distribution where also obvious. In the control site *Quercus prinus* dominated, but in the dusty site *Q. alba*, *Q. rubra* and *Liriodendron tulipifera* dominated. Thus, dust accumulation favours establishment of some species but limits others. Indeed, in dust-contaminated areas it is highly unlikely that acidophiles and conifers will persist (Brandt and Rhoades, 1972).

Seasonal variation in dust deposition is important as there are distinct differences in climate between seasons. Prajapati and Tripathi (2000) discovered that more dust was deposited on all plants during the winter (dry season) than in the summer and rainy seasons. In winter the physiological effect of dust on plants was more negative than in all the other seasons, when the climatic conditions resulted in lower dust deposition amounts. Armbrust (1986) found that, in the first week of testing dust effects on cotton plants, as much as 90% of the dust was removed from plants by rain and 46% was removed by wind in 2.5 days. The authors thus concluded that, under natural conditions, even if large quantities of dust can potentially be harmful to plants, the rapid removal of the dust by wind and rain make it highly unlikely that it would pose a major problem to cotton plant production (Armbrust, 1986).

1.2.5. Other impacts and indirect effects

1.2.5.1. Soil characteristics

Dust does not only affect the vegetation by deposition on aboveground parts, but also indirectly through the soil and root system (Ulrichs et al. 2008). The most interesting indirect plant responses are soil-mediated, and the changes within the soil depend heavily on the chemical composition of the dust pollution (Prajapati, 2012). Only after 10 years or more of dust pollutant accumulation would changes in the soil be observed, with the exception of industrialized point sources where surrounding areas are highly polluted (Saunders and Godzik, 1986). As a result, indirect effects are not easy to determine accurately as changes occur over a long time and are naturally subtle (Garner, 1994).

Particulate matter increases can drive changes in soil pH depending on the chemical composition of the pollutant. A pH range of 5.5 to 6.6 is the optimal conditions for most crop plants and any changes to soil pH that render it out of this range may affect the availability of essential nutrients for the plant (Ulrichs et al. 2008). Liming can have several effects on the soil. It can cause an increase in magnesium, calcium, and phosphates in the soil. Base saturation may also increase as well as solubility of some irons such as manganese, aluminium, and iron. However, over liming of the soil can result in copper, manganese, zinc and iron deficiencies (Brady, 1974). Dun (1983) noted dust from unpaved roads contained limestone material, which increased pH levels and resulted in some trace element deficiencies in plants.

Dust containing metals can have a toxic indirect effect on plants. Sing et al. (1997) observed that high lead concentrations were found in densely polluted urban areas near industrial waste disposal. The metal was found to inhibit nitrate reduction and also inhibited nitrogen fixation, ammonium assimilation, and nodulation within the root nodules of plants. However, the extent of the toxic effect depends on environmental factors and nutritional factors (e.g., high inorganic salt content can antagonize toxic effects) (Sing, 1997). Maina et al. (2015) evaluated the effect of cement dust on plants in the vicinity of a cement factory. They noted that heavy metal concentrations within plants increased 2–10-fold more in magnitude compared to in unpolluted soil. These high concentrations, especially for lead, exceeded the toxic limits within the plants (Maina et al. 2015). Airborne road dust may also affect biota by transporting elements such as Fe, Al, Cr, and Ni that enrich the surrounding soil (Rahul and Jain, 2014).

In another case, O'Connor (1983) found that plants that grew on poor substrata received more organic matter from road dust, which improved their condition (McCrea, 1984). Furthermore, particulate matter can directly affect the rhizosphere fungi and bacteria by influencing nutrient cycling, especially regarding nitrogen within the soil (Grantz et al, 2003).

Usually, particulate matter deposition would not alter the physical structure of the soil, because it makes up only a fraction of the topsoil volume (Ulrichs et al. 2008). When, however, particulate matter is mixed and deposited artificially in the soil for example by means of coal fly ash deposition or if natural dust storms bring forth a high concentration of particulate matter, it is likely that soil physical structure may be affected (Grewal et al. 2001). In the most extreme case, Krippelova (1982) found that in the vicinity of a magnesite factory, surface crust formed in grasslands and soil pH increased to as much as 9.5. Chaturvedi et al. (2013) measured soil moisture content, soil texture (clay, silt, and sand), pH, organic carbon and total nitrogen and phosphorus content between a site near a busy road, which was also near a cement factory and a site 40 km away (control). Only soil moisture content, clay, and total nitrogen were found to be statistically significantly different between the sites (Chaturvedi et al. 2013).

1.2.6. Dust sampling methods

Dust samplers were first developed when researchers began modelling and measuring dust emissions due to its potential impact on human health (Pietersma, 1996). Dust samplers can use active and passive dust sampling techniques, and sample dust flux horizontally or vertically (Kwata, 2014). Passive sampling depends heavily on wind conditions whereas active sampling relies on a suction operator to draw particles into the trap, which is why particle size is considered an important factor when choosing a method and for explaining results (Youseff et al. 2008).

Six of the most widely used aeolian dust samplers were mentioned by Goossens and Offer (2000). The samplers were as follows: The big spring number eight sampler (BSNE), the wedge dust flux gauge (WDFG), the suspended sediment trap (SUSTRA) and the modified Wilson and Cooke sampler (MWAC) were used to measure horizontal flux, and the marble dust collector (MDCO) and Sierra ultra-high volume dust sampler (SIERRA) to pick up vertical deposition flux (Kwata, 2014; Fig. 1.1).

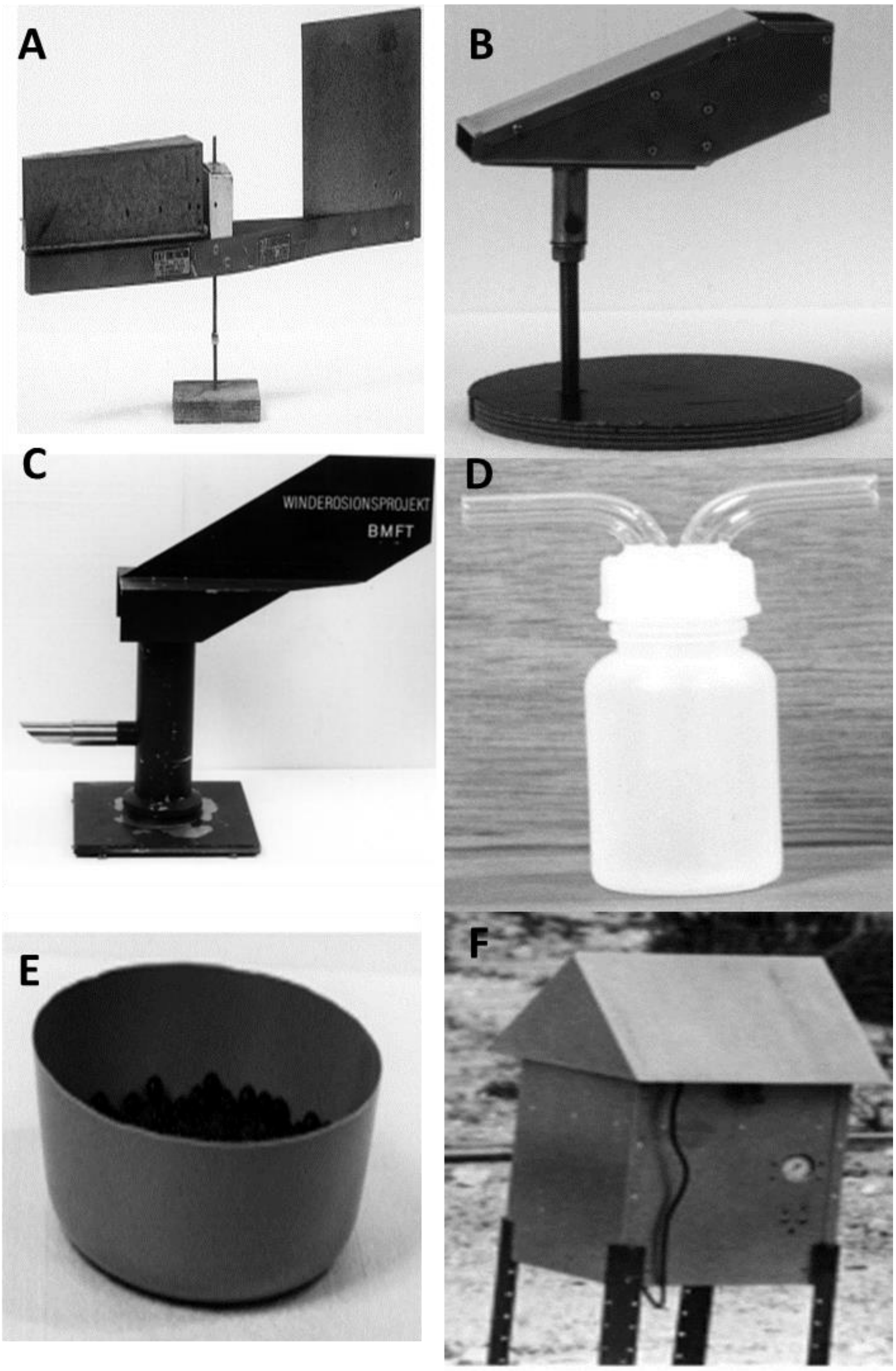

Figure 1.1. Photographs of the dust samplers (A) big spring number eight sampler (BSNE), (B) the wedge dust flux gauge (WDFG), (C) the suspended sediment trap (SUSTRA), (D) the modified Wilson and Cooke sampler (MWAC), (E) the marble dust collector (MDCO) and (F) the Sierra ultra-high volume dust sampler (SIERRA) (Goosens and Offer, 2000)

The most important aspect of any dust sampler is its efficiency, and this depends on a variety of factors (Goosens and Offer, 2000). The first factor is the particle size. It is important as it determines the inertia of the grains along with wind speed in relation to the collector. Larger particles need a higher wind speed to reach dust collectors but are more easily contained by traps. This is the complete opposite of finer particles. Another important factor is the trap inside the dust collector itself. The trap should be effective enough to not allow finer particles to leave the dust collector (Kwata, 2014; Goosens and Offer, 2000). The effect of time should not be underestimated. A dust-trap can become less efficient as the trap becomes more saturated over time. However active dust samplers become more efficient if the traps become saturated and the flow is automatically adjusted accordingly (Andrew et al. 2012). Finally, and perhaps the most important factor is the design of the collector. The size and shape of the collector determines the degree of obstruction (Goosens and Offer, 2000; Andrew et al. 2012).

In the context of human health, it can be important to measure and obtain inhalable, thoracic and respirable aerosol fractions in the air (Belle, 2018). These are often obtained by using cyclone samplers. In epidemiological studies, it can be crucial to evaluate respiratory disease risk when a workplace is exposed to dust (Kenny et al. 1999). Respirable dust samplers play a pivotal role in such monitoring (Belle, 2018). Nevertheless, each of the samplers hastheir own specific-use efficiency, pros and cons that are strongly dependent on particle size or wind velocity, or both.

The efficiency of three thoracic aerosol samplers with respect to particle aerodynamic diameter was tested by Gorner et al. (2017) in a vertical calm air chamber and horizontal wind tunnel. The efficiency of aerosol sampling varied with particle size and with particle phase. In a dry-state, particles tended to bounce off the wall of the samplers, whereas particles with moisture adhered to the internal surface of the samplers. A study conducted by Youssef et al. (2008) portrayed the importance of particle size and wind speed in a sediment trap's catch efficiency. At similar wind speed comparisons, the catch efficiency for an MWAC was extremely low for sand particles smaller than 50 μm compared to a Vaseline Slide's (VS) catch efficiency, which was as much as 92%. However, the complete reverse happened with bigger sand particles (Youssef et al. 2008). Other significant findings of the study indicated that the MWAC was more efficient at sampling sediments at higher wind speeds than the VS, however it was not as efficient as the VS was at sampling sediments at higher heights (Youssef et al. 2008).

The horizontal flux samplers, MWAC, WDFG, SUSTRA, and MDCO—listed here in order of how they were ranked with respect to their dust sampling efficiencies-all had variable efficiency because of factors such as variable wind speed, particle size, soil texture and time (Goosens and Offer, 2000). Thus, it is not necessary for sediment traps to be highly efficient for it to be useful. Any trap can be useful depending on the objective in mind, while considering the local surrounding conditions and the factors influencing dust-trap efficiency (Drew and Lippman, 1978).

1.2.7. Anthropogenic dust generation: management implications

Before understanding the relationship between ecosystem components and biodiversity the main issue would be classifying and defining what ecosystem services are. Ecosystem services result from the co-production of societies and ecosystems to provide certain benefits that people can use (Balvanera, 2016). In ecology one of the most unresolved questions is the specific nature of interdependencies between ecosystem functioning and the diversity or structure of biotic communities (Groot et al. 2010). When synergies and trade-offs need to be taken into account, ecosystem services can be an important decision-making tool that can be incorporated into policy targets to help manage the resources of the planet (ICSU et al. 2008; Balvanera, 2016). Quantification of ecosystem services is crucial, otherwise decisions, that determine the fate of the benefits terrestrial ecosystems may provide are made with little understanding of the outcomes (cost and benefits of ecosystem services) and consequences for stakeholders.

There are three main issues regarding the loss of soil material from unpaved roads. The first issue has to do with soil degradation, which can result in unsafe driving surfaces and high maintenance costs. Secondly, dust clouds obscure the vision of the driver in the vehicle behind, and the final issue is that dust has the potential to degrade the quality of life, e.g., through inhalation and ensuing respiratory issues, as well as eye irritation and potential blinding of both livestock and the herders that care for them (Jackson, 2015). Due to rising polluting activities such as mining, industrial processes and road dust pollution many plants close to the source have been threatened (Sett, 2017).

Minimal dust control in South Africa is being carried out on unpaved roads despite there being good options available (Jones, 2000). There are various management techniques to quell dust pollution, but they differ in practicality, cost, and effectiveness. For e.g., the amount of dust generated is proportional to vehicle speed and thus in some countries, speed reductions are an effective measure of dust control. Another option is the use of petroleum-based suppressants, but the product choice should consider the evaporation temperature, porosity of the road surface and the impacts on the environment (Succarieh, 1992). Generally, the use of chemical palliatives is more a short-term solution, while a more long-term solution would be sealing, although it should be noted that concrete or bituminous-sealed roads still produce some dust but in smaller quantities (Greening, 2011). Other short-term solutions are water and wetting agents and waste oils; however, the latter is not recommended in South Africa as the soil has a very high potential for ground water pollution. Other products that can be used in road dust control include industrial and plant wastes, molasses and tannin extracts (Greening, 2011; Jones, 2000).

To help control the quality of products, to avoid adverse worker health and to maintain the optimal performance of machines, Ahmad et al. (2006) implemented a dust control system in an electronics company that was divided into three main phases. The first phase is implemented before the dust control system can be introduced. It mainly focuses on identifying how severe (the level and distribution) the air pollution problem is within the production floor. The second phase focuses on identifying what the main causes of dust particles in the production floor are, by using management and planning tools. Lastly, the effectiveness of the solutions used in the second phase wasevaluated. After the implementation of the dust control system dust particles were reduced to 80.2 % compared to the previous state of the whole sub-area (Ahmad et al. 2006).

In recent years, for various applications such as global air quality and dust forecasting, there has been a rapid increase in the use of dust modules and implementation (Knipperz and Todd, 2012). Trivedi et al. (2009) used air quality modelling by means of a fugitive dust model to determine whether dust generated by mining contributed significantly to the air quality of the surrounding area 500 m away from the mine. Triparthy et al. (2015) similarly used AERMOD software to predict dust concentrations from nearby areas of a coal mine (personal dust exposure) to characterize dust dispersion from various sources and to monitor levels of dust from the mine at different operational areas. However, there can be various issues regarding the modelling of dust processes (Knipperz and Todd, 2012). Modelling the emissions of dust for example in North Africa can differ by as much as a factor of five. This is because of differences in 1) soil properties, 2) representation of peak winds and 3) dust emission parameterization, which can cause models to provide different results (Knipperz and Todd, 2012). These issues make it very difficult for agencies to implement the correct management strategies or the best possible environmental impact assessment (EIA).

One of the aims of this literature review is to inform readers of the impacts of anthropogenically induced dust generation on vegetation and other components of biodiversity, impacts that are often overlooked. This is especially important in dryland ecosystems, where there are typically higher levels of bare soil (low vegetation cover), and where upon disturbance strong winds can carry dust across their vast plains, affecting ecosystem health, fauna and flora, as well as human well-being. There isn't just one factor that can be singled out as the extent of the impact of anthropogenic-related dust depend on an interrelated complex of many factors, including dust source, weather conditions, plants species and their mitigation traits, and the physical, chemical and microbial characteristics of dust. A particular plant community can either respond positively, negatively or not at all, but it would depend on which of these factors predominate over time and space. The other aim of the review is to provide some useful information that environmentalists and conservationists can utilize in order to manage plant communities that experience issues with anthropogenic-related dust. In sum, in a world of rapid environmental change, dust generation by humans must not be ignored, especially not in arid areas, as it will most likely negatively affect fauna and flora, as well as human well-being as future conditions become drier. On the upside, it is clearly an aspect that could be managed effectively.

1.3. The Current Thesis

1.3.1. Problem Statement

The Nama-Karoo biome in South Africa is the third largest biome and has the harshest climatic conditions. Limited water resources and extreme temperatures, in combination with young soils leads to low plant biomass (Ellis, 1988). The Succulent Karoo biome is known for its diverse soil geology and high plant endemism and biodiversity (Dojani et al. 2013). With already low vegetative cover, the movement of dust onto plants could further affect their health. Little research has been conducted assessing the effects of dust from unpaved roads on the fitness of plant species across the globe, the semi-arid Nama-Karoo and Succulent Karoo being no exception. This means that there is minimal information on how dust from unpaved roads affects plant communities in these biomes, information that could be useful toward conservation planning or environmental impact assessments. Indeed, increasing pressures on the environment in these drylands are likely as development here reaches new heights (Walker et al. 2018). More information is therefore needed to help mitigate the damage to Karoo vegetation from anthropogenic activities. The physical characteristics of dust, e.g., dust load and particle size can vary depending on the source from which dust is generated. It is thus essential to determine these physical characteristics first to evaluate the potential impacts of dust on plant structure and function, and subsequently an overlooked aspect of biodiversity in this area, the soil microbiome.

1.3.2. Aim

This research aimed to evaluate dust characteristics generated by vehicles on gravel roads along a distance gradient from the road, and its subsequent effects on Karoo plant function and microbial diversity along this gradient at two Karoo sites with varying physical characteristics.

1.3.3. Objectives and specific research questions

Objective 1: To determine the dust load and particle size with increasing distance from unpaved roads at selected sites in the karoo.

1.1. What are the physical characteristics of dust, e.g., particle size and dust load?

1.2. What are the relationships between dust load and particle size with distance from unpaved roads?

Objective 2: To determine the impacts of road dust on leaf structure and function of different plant functional types, both sampled at varying distances from the road, by comparing leaf specific area (SLA), leaf stable carbon and nitrogen isotopes and metal ion deposits for both shrubs and a succulent species.

2.1. What is the distribution of metal ions in and on plant leaves along the distance gradient?

2.2. How do functional traits such as SLA, and C and N isotopes differ along the distance gradient?

2.3. What is the relationship between metal ions and plant functional traits along the distance gradient?

2.4. Does dust affect the functional traits of the two species within each site to the same extent?

2.5. What is the leaf functional responses of shrub and succulent species to dust?

Objective 3: To determine the effect of dust generated from unpaved roads on microbial dispersion patterns, by comparing the species richness and assemblage composition of fungi and bacteria along with increasing distances from the unpaved road.

3.1. Are there differences in the dust's microbial composition with distance from the road, and how does it differ from the road's soil surface and the soil in the matrix?

3.2. How does species richness for bacteria and fungi differ?

3.3. Which factors are strong determinants of microbial composition and richness in the two sites?

1.4. Thesis Structure

Chapter 1: Includes the general introduction, literature review and the project aim, objectives and research questions of the current thesis. The literature review is based on the impact of anthropogenic dust generation on ecosystem health, whereas the current thesis placed emphasis on the impacts of dust generation from unpaved roads in two Karoo landscapes.

Chapter 2: The physical characteristics (dust load and particle size) and the spatial deposition of dust, that was generated from unpaved roads in two Karoo landscapes were examined.

Chapter 3: The effects of dust from unpaved roads on plants across semi-arid Karoo landscapes were determined by comparing carbon and nitrogen isotopes, SLA and metal ion deposition.

Chapter 4: The impacts of dust generated from unpaved roads in the Karoo on soil and sampler microbial diversity and composition were analysed.

Chapter 5: The Synthesis combines the ideas obtained from chapter 1 to chapter 2 into a conclusion.

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Chapter 2: Examining the physical characteristics and spatial deposition of dust generated from unpaved roads in two Karoo landscapes

2.1. Introduction

Dust can be considered a threat to the environment and human well-being. When vehicles drive on unpaved roads, they blow dust into the atmosphere. The suspended particles will fall on the leaves of plants near the emission zone and pose a direct or indirect threat to the survival or productivity of plants (Shah et al. 2017). Dust can physically injure plants through sandblasting, resulting in tissue damage, or indirectly alter soil pH, leaf temperature and photosynthetic rate (Lewis et al. 2017; Shah et al. 2017). Although roads are obviously important, they nonetheless, from an environmental standpoint, alter plant and animal dynamics, introduce exotic species, change available resource levels, and alter nutrient flows (Mullerova et al. 2011).

Fine dust particle sizes are either from soot or diesel whereas coarser dust particles come from road dust and soil (Rai et al. 2010). Studies indicate that for finer particles a lower concentration of dust is necessary to cause an effect on plants, whereas higher dosages are required for more coarse particles (Sett, 2017). Sedimentation of finer particles has a much greater effect on the lower leaf surface, while coarser particles affect the upper leaf surface more (Thompson et al. 1984). Nevertheless, resuspended road dust, of particular concern in this study, is mainly recognized as a source of coarse particles (Vallius et al. 2005).

Shah et al. (2017) discovered that coarse road dust can alter a plant guard cell's osmotic potential, resulting in shrinkage of the guard cells and ultimately reducing the transpiration rate of the plants. Rai et al. (2010) found that on leaves a proportion of 75% of total deposited dust consisted of particles with diameters ranging between 2.5 and 10 µm. These finer particles clog stomata, affect gaseous exchange, photosynthesis, leaf temperature, respiration and water retention. Ecosystems may, therefore, be adversely affected, and so obtaining a better understanding of how dust deposition may look across landscapes would help inform both road design and conservation management.

To fully gauge the influence of dust on vegetation, it is important to understand that impacts differ as they depend on the interaction between the properties of the vegetation and the dust particle size (Rahul and Jain, 2014). Smaller vegetation elements (such as fine leaves) are better at removing particles from an air stream than are larger elements. Kaler et al. (2016) determined dust accumulation on four different plant species next to a national highway at two distances: 0–5 m and 5–10 m. Dust accumulation on leaf surfaces was significantly higher closer to the road and varied significantly between species due to differences in leaf characteristics, bark and twigs (Kaler et al. 2016). Swain et al. (2016) measured the effect of dust pollution on plant chlorophyll content with varying distances away from the source. Where dust loads were high near the pollution source, plant chlorophyll was severely degraded, but the negative effect gradually diminished for plants situated further away from the source (Swain et al. 2016). A similar trend, that where dust deposited closer to the road and where the negative effect diminished with distance away from the road, was found by Avon et al. (2013), Mullerova et al. (2011) and Gunn (1998).

In addition to particle size and flow distance, dust load is an important factor. It was shown that only at a relatively higher dust load did the direct physical effects of mineral dust on vegetation became apparent (Farmer, 1993); however, depending on the chemical composition of the mineral dust, chemical and indirect effects may become evident at much lower dust loads. For example, cement dust or particulate sulphates and nitrates can have indirect effects on ecosystems at only 2 g m^{-2} (Farmer, 1993). An experiment conducted by Raja et al. (2014) used different fly ash deposition amounts and evaluated the effects on plants. They found that for fly ash any amounts from 0.5 g m^{-2} and above significantly reduced plant stomatal conductance, transpiration and photosynthesis. There are many gaps in the literature relating to the impacts of road dust on vegetation.

The Nama and Succulent Karoo are known for their harsh environmental conditions but are diverse in terms of plant species, especially the Succulent Karoo; thus, it is useful to understand what the impacts of road dust on these ecosystems might be. Also, both of these biomes are poised for development, which ranges from activities such as fracking to uranium mining, thus resulting in more unpaved roads. The minimal information available on how dust from unpaved roads affects biota in these two biomes, however, makes it difficult to include road dust as a factor in conservation planning and environmental impact assessments. The physical characteristics of dust, e.g., dust load and particle size, can vary depending on the source from which dust is generated. It is thus essential to determine these physical characteristics first in order to evaluate the potential impacts of road dust on plant structure and function. The overarching aim of this chapter, then, is to quantify for the first time, the pattern at which dust load and particle size along a distance gradient from unpaved roads settles, and in so doing provide useful information to aid environmental and conservation planners in road dust management in these semi-arid ecosystems. I predict that at both sites, higher dust loads and more coarse particles will settle at distances closer to the road (0-20 m and 21-100 m) whereas lower dust loads and finer particles will settle at distances further away from the road (101-400 m and 401-1000 m).

2.2. Materials and methods

2.2.1. Site description and experimental design

Sampling took place at two sites with varying biophysical characteristics in the Karoo biomes of South Africa. Two large (7–8 m wide) and often-used gravel roads were selected. The first road was within the Square Kilometre Array (SKA) radio astronomy reserve near Carnarvon, in the Northern Cape Province of South Africa, and falling within the Nama Karoo biome. The second road was adjacent to the Wolwekraal Nature Reserve near Prince Albert, in the Western Cape Province, falling within the Succulent Karoo biome (Table 2.1). The soils of both biomes have little organic matter and are poorly developed and the windblown movement of sand can affect its biota (Milton and Dean, 1999). At the SKA site, the landscape surrounding the gravel road consists of low shrubland on flat plains (Fig. 2.1A), with scattered dolerite mesas and buttes. At Wolwekraal, the area adjacent to the road is undulating and trees are interspersed with low shrubland because the study site selected at Wolwekraal was nearer a large drainage line (Fig. 2.1B). The average climate conditions of the SKA reserve and that of Wolwekraal are remarkably similar; so too wind directions and speeds (Table 2.1). The predominant wind direction at both sites are W-NW, mostly at speeds between 5.6–8.7 m s^{-1} along those directions. Finally, although not formally established, traffic at Wolwekraal is estimated to be considerably higher than at SKA, with the latter's road only used by SKA operators in the area, but the former being a public road.

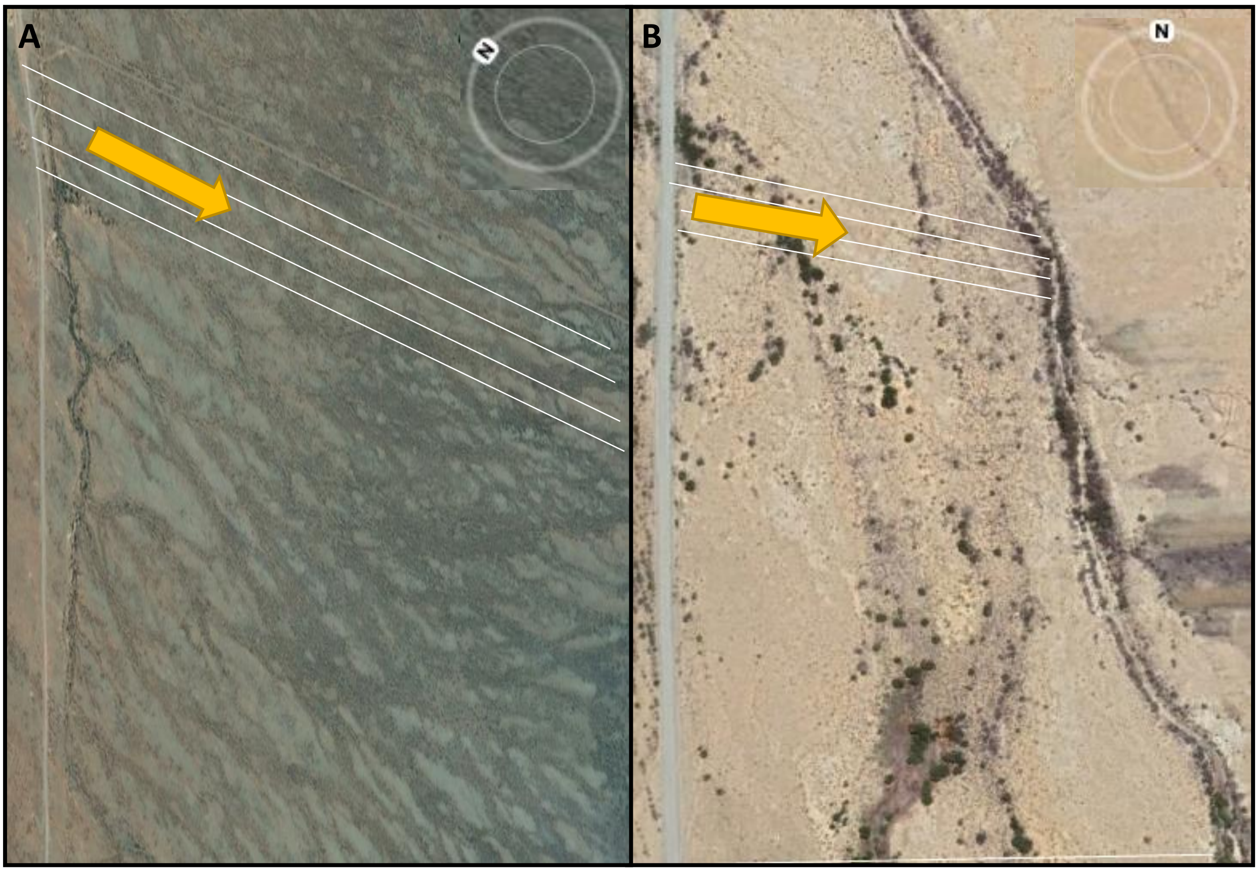

Figure 2.1. Satellite images of the two study areas: (A) SKA and (B) Wolwekraal, portraying the different vegetation structure and bare soil characteristics and four transects (white lines) where sampling took place. The yellow arrows indicate the dominate wind direction at both sites. Source: Google, Inc.

Table 2.1**.** Summary of sampling site characteristics, including climate, the target species to sample, and the gravel roads in question with prevailing wind directions and speed.

	Square Kilometre Array Reserve	**Wolwekraal Nature Reserve**
Nearest town	Carnarvon, Northern Cape	Prince Albert, Western Cape
Biome	Nama-Karoo	Succulent Karoo
Sampling Species	*Rhigozum trichothomum* (shrub)	*Pteronia pallens* (shrub)
	Pteronia glauca (shrub)	*Ruschia spinosa* (succulent)
Mean Annual Precipitation	±200 mm	±200 mm
Mean Max. Temperature	24 °C	23 °C
Mean Min. Temperature	9 °C	10 °C
Prominent Wind Direction	W-NW	W-NW
Average Wind Speed	5.6-8.7 m s^{-1}	5.6 m s^{-1}
Topography	Flat	Slightly undulating
Gravel Road Diameter	7 m	8 m
Car and Truck Access	YES	YES
Number of Vehicles Passing p/h	Estimated at 0–5 per hour	Estimated at >10 per hour
Vegetation height	±0.1–1.5 m	±0.1–4.0 m
Vegetation cover	Low-growing shrubs, few succulents	Low-growing shrubs, many succulents, trees interspersed

The main wind direction was considered when designing the sampling layout. Sampling at both study sites took place from the road into the interior of the properties, along four line transects that were each spaced into distance categories, namely D1 = 0–20 m, D2 = 21–100 m, D3 = 101–400 m and D4 = 401–1000 m. Due to the smaller sampling area at Wolwekraal Nature Reserve, sampling took place in the same distance categories as at SKA but closer to the lower limits of each distance category (i.e., D1 = 0–5 m, D2 = 21 m, D3 = 101 m and D4 = 401 m), as opposed to at the Square Kilometre Array Reserve, where sampling mainly took place closer to the upper limits of each distance category (i.e., D1 = 0–20 m, D2 = 100 m, D3 = 400 m and D4 = 1000 m).

A MWAC (modified Wilson and Cooke) dust sampler was used to obtain dust loads from the roads at both sites. Four MWAC dust samplers were placed next to two neighbouring plant species (evergreen species, Table 2.1) within each distance category of a single transect

(Fig. 2.2). Two dust samplers, one at the top (1.3 m high) and one at the bottom (ground level) of a steel rod were placed next to each species. Thus, a total of 16 dust samplers (eight bottom and eight top) were used per distance-based transect, leading to a total of 64 dust samplers and 32 plants sampled per site (Lewis et al. 2017).

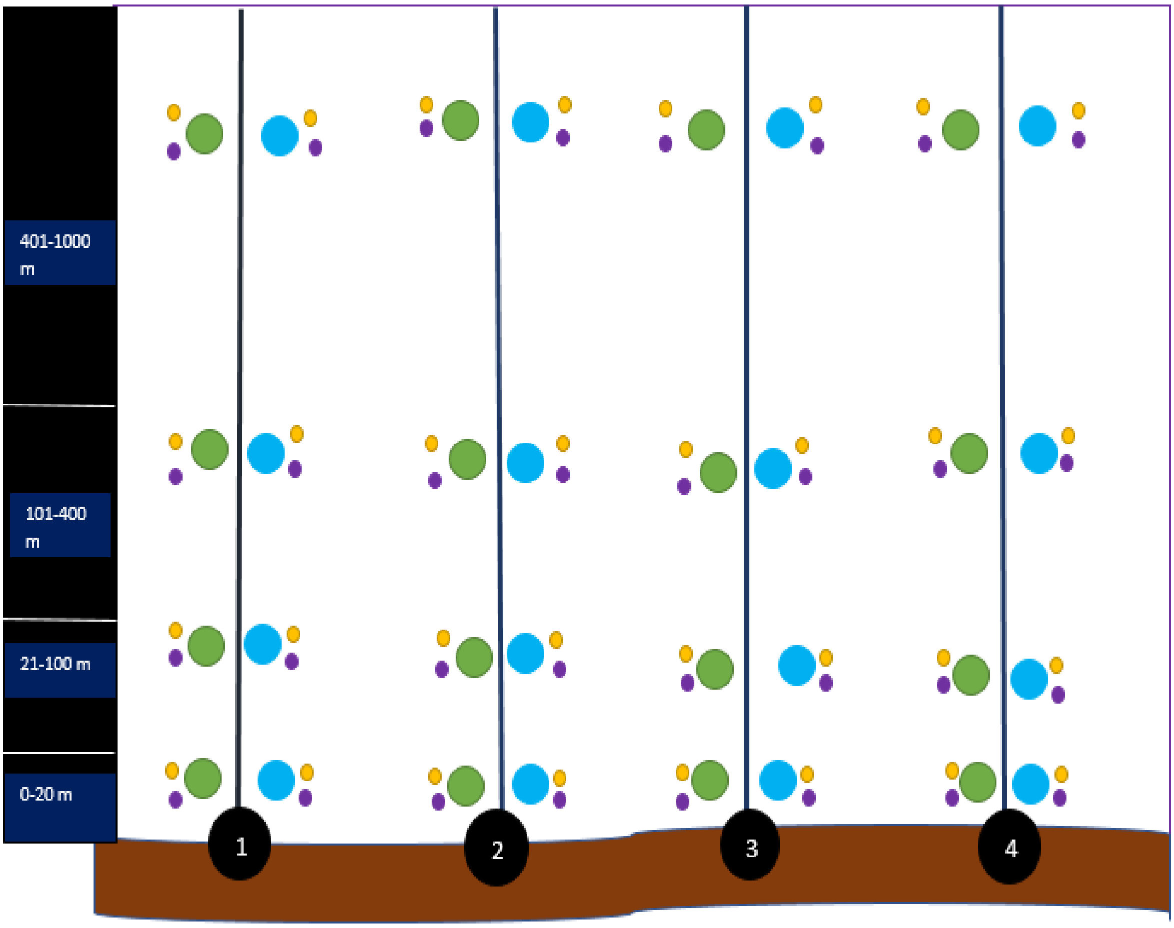

Figure 2.2. A sketch portraying the experimental design of this study. The entire sampling area was divided into four distance categories (D1: 0–20m, D2: 21–100m, D3: 101–400m and D4: 401–100m) with sampling replicated along four line transects (Bottom, 1–4). Within each distance category, a line transect consisting of 16 MWAC dust samplers, eight at the top (yellow circles) and eight at the bottom (purple circles) were used to collect deposited dust next to two plant species, Species 1 (green circles) and Species 2 (blue circles).

2.2.2. Dust sampling and approach

When dust samplers were positioned next to each plant, the adjacent topsoil was also sampled from the soil surface to at a depth from 5 to 10 cm using a shovel. The dust samplers were left in the field for 60 days, between October and December 2018 (the windy season), and the inlet tube of each sampler directly faced the main wind direction measured on-site.

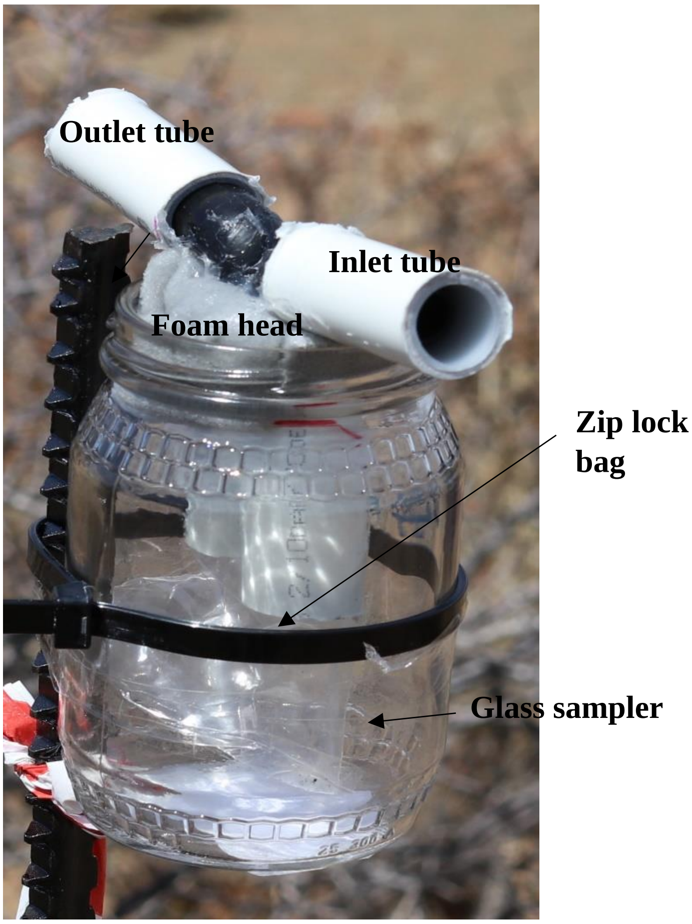

Fig 2.3. An image of the modified Wilson and Cooke (MWAC) dust sampler (adapted from Goosens and Offer, 1999). The foam head was tightly sealed with silicon to prevent any potential rain or dust from entering through the top.

The MWAC dust sampler is a simple piece of equipment that consists of a glass sample bottle with a 3-cm-thick foam head that covers it (Fig 2.3). From the foam's head protrudes two plastic tubes, the inlet tube that allows air into and the outlet tube where air flows out of the bottle (Goosens and Offer, 1999). A zip-lock bag captures the dust that flows through the inlet tube into the sampler.

After eight weeks the dust samplers were collected, the foam head was removed to obtain all of the marked zip-lock bags that contained the deposited dust. The samples were oven-dried open at 45 °C until a constant weight was achieved. All dust sample weights were measured to two decimal places using an electronic balance (RADWAG, WPS 1200/C/2).

Particle size measurements were done by suspending a tiny scoop of dust in a single distilled water droplet using a pipette (Lewis et al. 2017). The suspension was mounted beneath a cover slip on a microscope slide. With the help of the ZEISS app, the maximum diameter of 30 randomly selected particles were measured to two decimal places in micrometres using a microscope (ZEISS Discovery V 8).

2.2.3. Statistical analyses

Statistica 13.5.0.17.msi was used to conduct all statistical analyses. The data were tested for normality by means of a Shapiro–Wilk test. Data that were non-normally distributed were transformed using $\log_{10}$. A Post hoc Tukey HSD test was used to determine significant differences of dust loads and particle size between distance categories. A Pearson correlation was used to determine the strength of the relationship between particle size and dust load along the distance gradient.

2.3. Results

2.3.1. Dust load

Samples were collected after two months; the dust load differed between the two sites and between the two sampling heights on each sampler. At Wolwekraal an average of 0.057 (± 0.05) g cm^{-2} was found, while at SKA 0.427 (± 0.83) g cm^{-2} was found. At both sites dust load was always higher near the ground compared to 1.3 m above the surface — within each distance category. Variability at Wolwekraal was greater than at SKA and greater near the ground compared to 1.3 m above the surface at each site (Fig. 2.4C, D). In every distance category (D1–D4), average dust loads at SKA were more than the average

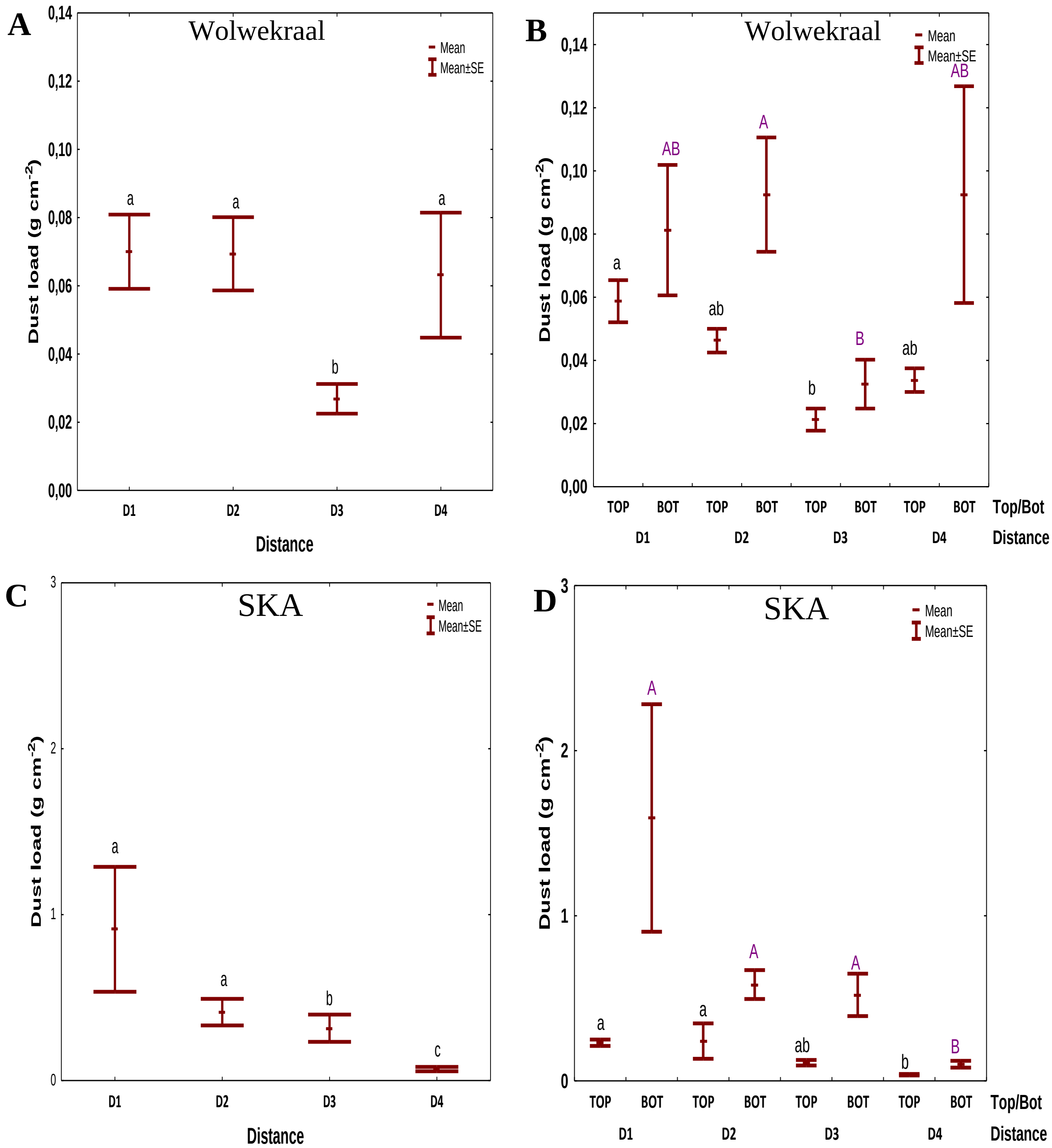

Figure 2.4. (A) Mean dust load, including all samples from the top and bottom, at four distance categories: D1 (0–20 m), D2 (21–100 m), D3 (101–400) and D4 (401–1000 m), at Wolwekraal. (B) Mean dust load from the separate top and bottom samples at Wolwekraal. (C) Mean dust load, including all samples from the top and bottom, at four distance categories: D1 (0–20 m), D2 (21–100 m), D3 (101–400) and D4 (401–1000 m), at SKA. (D) Mean dust loads from the separate top and bottom samples at SKA. (A,C) Small letters show statistical significance in mean dust among distances and different letters indicate significant difference at α = 0.05. (B,D) Small letters show statistical significance in mean dust load among distances at the top and different letters indicate significant difference at α = 0.05, whereas capital letters show statistical significance in mean dust load among distances at the bottom and different letters indicate significant difference at α = 0.05.

dust loads captured at Wolwekraal; however, the difference was greatest at the distance closest to the road (D1) and became gradually smaller away from the road (Fig. 2.4A,B).

At Wolwekraal (Fig. 2.4A) dust load at D3 was significantly lower than the dust loads at all the other distance categories (D1, D2, and D4), but differed most significantly ($p < 0.001$) from D1. The top dust samples only displayed a significant difference between D1 and D3 ($p = 0.020$), but at the bottom, it was D2 and D3 that was significantly ($p = 0.034$) different in their dust load (Fig. 2.4B).

At SKA (Fig. 2.4C), dust loads decreased with distance from the road. D4 differed significantly from all the other distances but differed most significantly ($p < 0.001$) from D1. Dust loads between D1 and D3 also differed significantly ($p = 0.027$). D4 differed significantly ($p < 0.001$) from D1 and from D2 ($p < 0.001$) at the top and at the bottom, D4 differed significantly from all the other distances but differed most significantly ($p < 0.001$) from D1 (Fig. 2.4D).

2.3.2. Particle size

After 30 random particles per sample were measured, a difference in particle size was found between the two sites and the two sampling heights. At Wolwekraal an average of 85.44 (± 58.82) µm was found, while at SKA an average of 143.82 (± 80.75) µm was found. At both sites, particles were always larger near the ground compared to 1.3 m above the surface—within each distance category. At Wolwekraal, the mean particle size displayed an inconsistent pattern among distances (D1 (84.12 µm) < D2 (102.41 µm) > D3 (62.79 µm) < D4 (92.43 µm)) (Fig. 2.5A). On the other hand, SKA displayed a consistent smaller mean particle size with distance (D1 (197.18 µm) > D2 (140.30 µm)> D3 (133.99 µm)> D4 (103.79 µm)) (Fig. 2.5B).

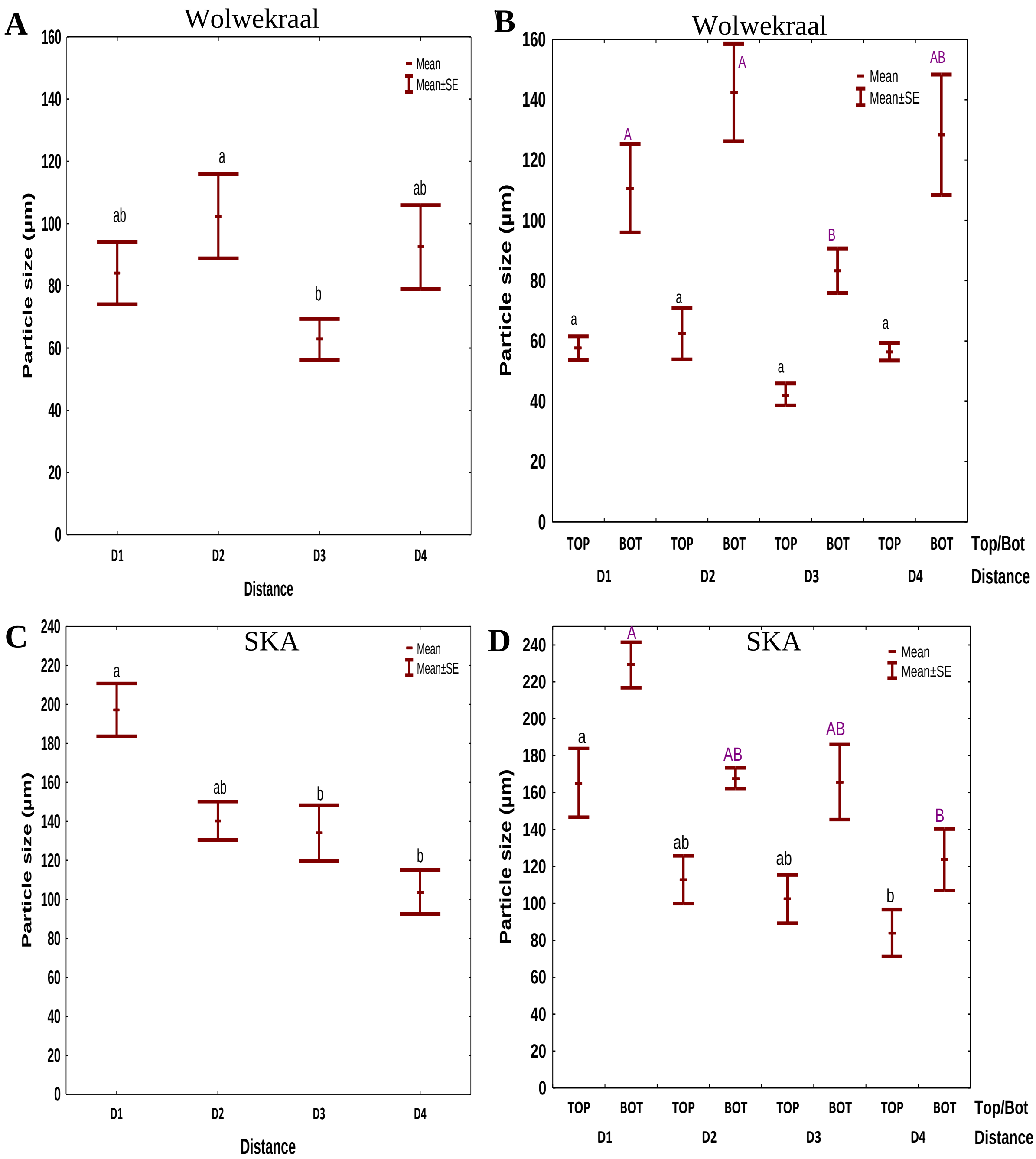

Figure 2.5. (A) Mean particle size, including all samples from the top and bottom, at four distance categories: D1 (0–20 m), D2 (21–100 m), D3 (101–400) and D4 (401–1000 m), at Wolwekraal; (B) Mean particle size from separate top and bottom samples at Wolwekraal; (C) Mean particle size, including all samples from top and bottom, at four distance categories: D1 (0–20 m), D2 (21–100 m), D3 (101–400) and D4 (401–1000 m), at SKA; (D) Mean particle size from separate top and bottom samples at SKA; (A,C) Small letters show statistical significance in mean particle size among distances and different letters indicate significant difference at α = 0.05; (B,D) small letters show statistical significance in mean particle size among distances at the top and different letters indicate significant difference at α = 0.05, whereas capital letters show statistical significance in mean particle size among distances at the bottom and different letters indicate significant difference at α = 0.05.

At Wolwekraal (Fig. 2.5A), only D2 and D3 differed significantly ($p = 0.049$) regarding their particle size with D2 higher than D3 but neither different from D1 and D4. None of the distances differed significantly from one another in terms of their mean particles size at the top, but similarly to dust load it was also D2 and D3 that differed significantly ($p = 0.021$) from one another at the bottom (Fig. 2.5B).

At SKA it was both D3 ($p = 0.015$) and D4 ($p < 0.001$) that differed significantly from D1 comparing the pooled sample (Fig. 2.5C). For both the top ($p = 0.0057$) and bottom ($p < 0.001$) it was only D1 that differed significantly from D4 (Fig. 2.5D).

2.3.3. Particle size classes

After the particle size distribution was measured, the size class of all particles were determined and expressed in proportion to each other. The selection of particle size classes was based on the Krumbein phi scale, a modification of the Wentworth scale. The proportion of particles in the different size classes were determined for Wolwekraal and SKA (Fig. 2.6), between distance categories (Fig. 2.7), and between the top and bottom samples (Fig. 2.8)

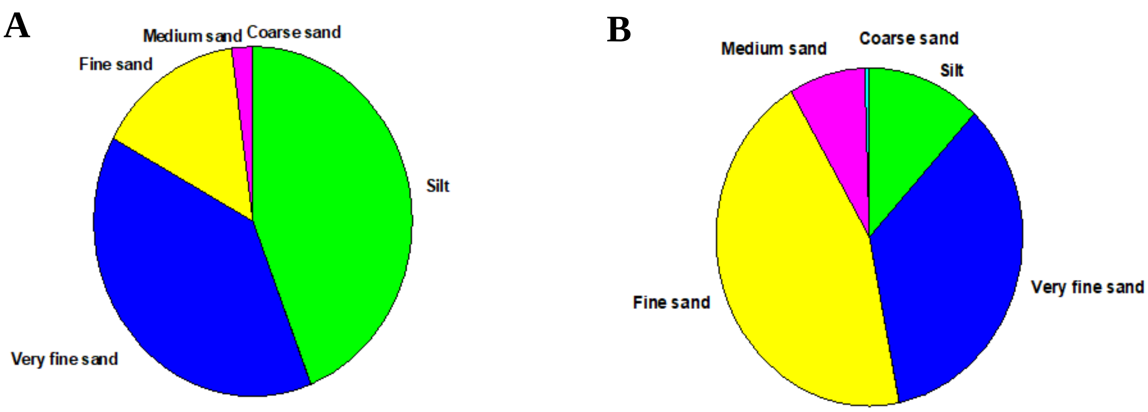

Figure 2.6. The general pattern of particle size class distribution in dust samples at Wolwekraal (A) and SKA (B), including all distances and top and bottom samplers. The Krumbein phi scale uses particle size diameter ranges of: 3.9–62.5 µm to classify silt, 62.5–125 µm to classify very fine sand, 125–250 µm to classify fine sand, 0.25–0.5 mm to classify medium sand and 0.5–1 mm to classify coarse sand.

For Wolwekraal (Fig. 2.6A), silt (44%) formed the largest proportion of particle size class, followed very fine (39%) and fine sand (15%) with a very small proportion consisting of medium sand (2%). However, in SKA the proportion of silt (12%) was less dominant and the medium sand (8%) proportion was more compared to Wolwekraal. Fine sand (45 %), followed very fine sand (35%) were the most dominant particle size classes (Fig. 2.6B).

At Wolwekraal the three dominant particle size classes, silt, very fine, and fine sand all displayed an inconsistent pattern in their relative proportions as distance increased from the road to the interior Fig. (2.7A–D). The same effect was present for the less dominant medium sand particle class (Fig. 2.7A–D).

In contrast, at SKA (Fig. 2.7E–H) there was a gradual decrease of the proportion of coarse particle classes (fine sand) and an increase of finer particle size classes (silt and very fine sand) with distance. Although the proportion of the larger particle size classes such as fine sand gradually decreased with distance from the road the one exception was medium sand, where there was an increase in its proportion from D2 to D3 (Fig. 2.7E–H).

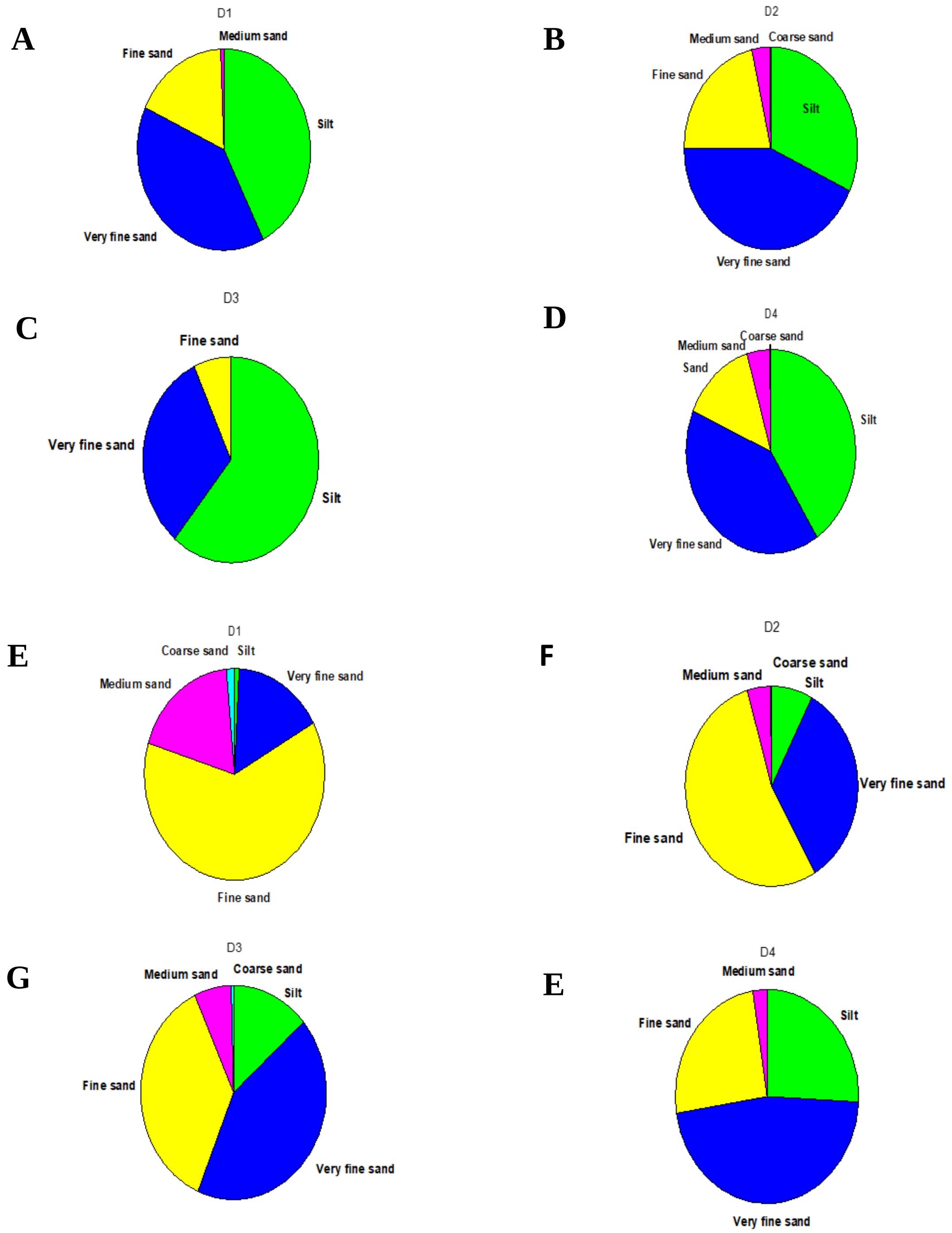

Figure 2.7. (A) The particle size class distribution at Wolwekraal from D1 (0–20 m); (B) the particle size class distribution at Wolwekraal from D2 (21–100 m); (C) the particle size class distribution at Wolwekraal from D3 (101–400 m); (D) the particle size class distribution at Wolwekraal from D4 (401–1000 m); (E) the particle size class distribution at SKA from D1 (0–20 m); (F) the particle size class distribution at SKA from D2 (21–100 m); (G) the particle size class distribution at SKA from D3 (101–400 m); and (H) the particle size class distribution at SKA from D4 (401–1000 m).

In samples from the top sampler at Wolwekraal (Fig. 2.8A), silt was the dominant particle size (72%) class followed by very fine sand (27%); however, at the bottom (Fig. 2.8B) very fine sand formed more than 50% (51%) of the particle size class proportion.

At the top samplers in SKA (Fig. 2.8C) very fine sand (44%) followed by fine sand (33%) were the dominant particle size classes. Fine sand (56%) followed by very fine sand (26%) dominated at the bottom (Fig. 2.8D). At the bottom in SKA medium sand (13%) formed a much larger proportion of the particle size class compared to the bottom in Wolwekraal (4%), with a similar finding between the top of SKA and the top of Wolwekraal.

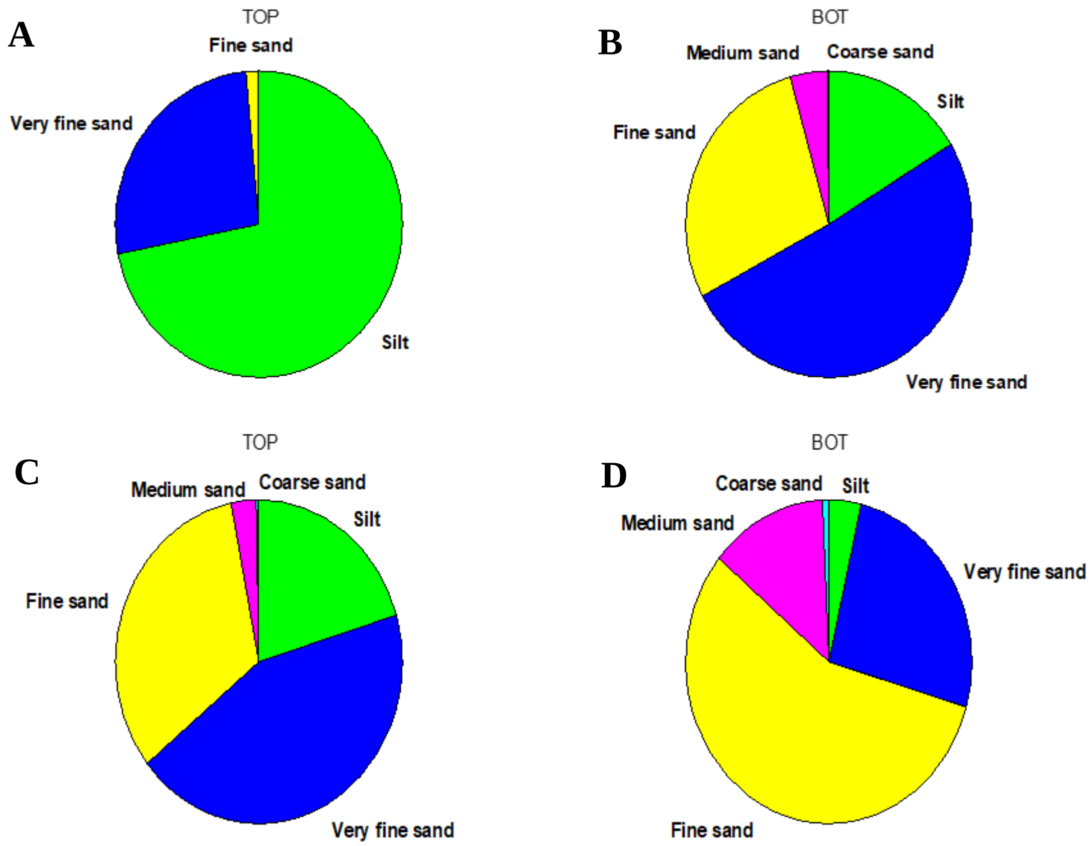

Figure 2.8. The distribution of particle size classes at Wolwekraal and SKA from both top and bottom samplers. (A) the distribution of particle size classes at Wolwekraal from top samplers; (B) the distribution of particle size classes at Wolwekraal from the bottom; (C) the distribution of particle size classes at SKA from the top; and (D) the distribution of particle size classes at SKA from the bottom.

2.3.4. Correlation between dust load and particle size

A Pearson's r correlation was conducted to determine the strength of the relationship between dust load and particle size along a distance gradient.

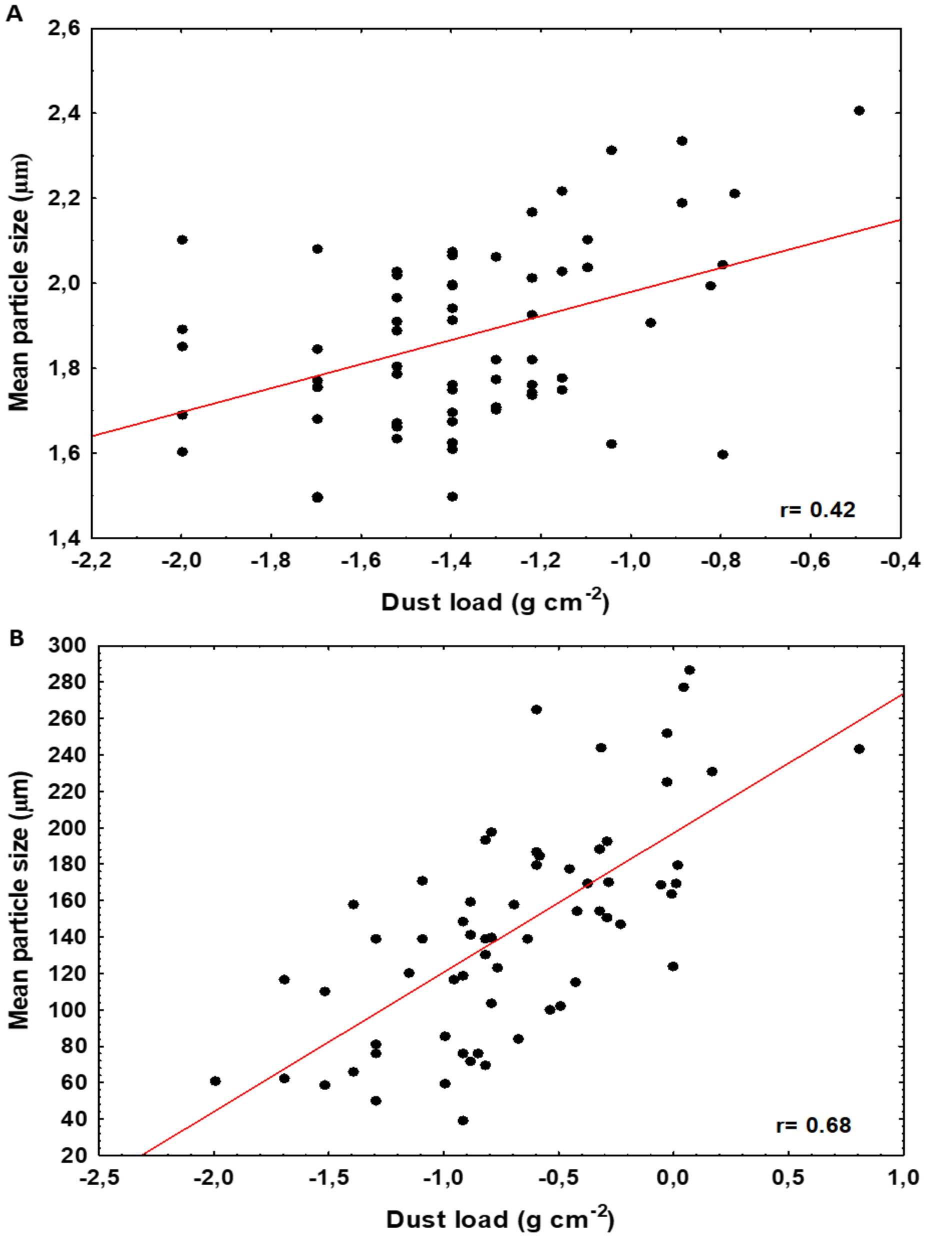

Figure 2.9. (A) Scatterplot to display the relationship between mean particle size and dust load with distance at Wolwekraal; and (B) scatterplot to display the relationship between mean particle size and dust load with distance at SKA. Negative values for dust loads were due to the normalized data using the $\log_{10}$ transformation.

At SKA a moderately strong positive (r = 0. 68) relationship was found between dust load and particle size (Fig. 2.9B), but at Wolwekraal a moderately weak positive (r = 0. 42) relationship was found between dust load and particle size (Fig. 2.9A)

2.4. Discussion

Very few studies in South Africa have tried to evaluate the physical characteristics of dust generated along a small-scale gradient from a gravel road and, as far as can be determined, none were conducted within the environmentally stressed Karoo region. A MWAC dust sampler was used to capture the horizontal dust flux generated from unpaved roads. The samplers captured on average more dust at SKA (0.43 (± 0.83) g cm^{-2}) than at Wolwekraal (0.06 (± 0.05) g cm^{-2}), including all samples from the top and the bottom (surface) (Fig. 2.4). Waza et al. (2019) used multiple dust samplers (passive and active samplers), including the MWAC dust sampler, to sample atmospheric dust. The MWAC sampler was the most efficient among all of the dust samplers and captured a maximum and minimum dust flux of 0.13 g cm^{-2} d^{-1} and 0.6 x 10^{-6} g cm^{-2} d^{-1}. When comparing the average dust load in their study to the average dust load captured at SKA (0.003 g cm^{-2} d^{-1}) and Wolwekraal (0,001 g cm^{-2} d^{-1}), the mean dust loads captured at both sites were between these maximum and minimum dust flux values. It should be noted that according to Goosens and Offer (2000) a sampler's design is the most important factor that affects its efficiency. The diameter of the inlet and outlet tubes used in this study was approximately 29 mm whereas in the study of Waza et al. (2019) it was 8–10 mm. The difference in the diameters of the MWAC samplers' tubes between the two studies could possibly play a role in the respective samplers' efficiency to capture dust.

Mean dust loads were highest closest to the road at both sites. A mean dust load of 0.02 g cm^{-2} d^{-1} was captured at SKA whereas at Wolwekraal a mean dust load of 0.001 g cm^{-2} d^{-1} was captured 0-5 m from the road. This finding was similar to the findings of other studies, showing dust loads could differ even when distances between samplers were small. For example, Kaler et al. (2016) found that irrespective of species or sampling season, plants within 0–5 m from the road had more deposited dust on their leaves (1.3 x 10^{-6} g cm^{-2} d^{-1}) than plants 5–10 m away (7.6 x 10^{-7} g cm^{-2} d^{-1}). Kaler et al. (2006) however, measured the average dust load that deposited on leaves during the dry and rainy seasons, whereas in this study sampling took place only during the dry season when wind speed is the highest and when there were no rain events. This seasonal difference could result in significant losses in deposited dust on leaves. For example, Rai and Panda (2014) found significant differences in dust loads deposited on *Ficus bengalensis* during the dry season (2.0 x 10^{-5} g cm^{-2}) compared to the rainy season (1.4 x 10^{-6} g cm^{-2}). In the dry seasons when dust loads were higher a greater physiological negative effect (e.g., a decrease in chloroplast amounts and photosynthesis) in plants were observed (van Heerden et al. 2006; Prajapati and Tripathi, 2000). Swain et al. (2016) determined the dust loads deposited on the leaves of six plant species that grew 500 m, 1250 m and 2000 m away from a sponge iron plant. Dust load on all of the species was higher at 500 m compared to the other two distances. The plants that grew in the 500 m range had higher rates of chlorophyll degradation compared to the other distances, which resulted in an overall lower photosynthetic capacity of those plants.

Walker and Everett (1987) made a similar finding but with different distance categories in Alaskan taiga and tundra. They also used unpaved roads as the dust pollution source. Thus, dust has the potential to reduce plant fitness by decreasing the overall photosynthetic performance of plants closest to the road to a greater extent than plants at distances further away from the road. This should be especially true in the dry season when rain cannot wash the dust from leaf surfaces.

This pattern of consistently decreasing dust load with increasing distance from the source was also evident at SKA. One of the objectives of Gunn (1998) was to measure the dust loads at various distances from a gravel road and found that there was an 80%–99% decrease in dust loads moving from the 0 to 30 m inward zone, and an even greater decrease between 0 and 100 m. Walker and Everett (1987) not only determined dust load between three zones along a distance gradient from a gravel road, but also analysed the particle size classes along this distance gradient. A consistent decrease in both dust load and particle size with distance away from the gravel road was also displayed at SKA, hence the moderately strong correlation between particle size and dust load that was found at this site. This pattern was supported by the findings of Walker and Everett (1987). They found that when dust load decreased from 10 to 30 m and from 30 to 100 m the particle size class distribution at these distances changed from 70 % sand (10 m) to 70% silt and clay (30 m), and to 90% silt and clay 1000 m. The proportion of finer particle sizes such as silt was greater at 1000 m in their study compared to what was found at SKA at 1000 m. The dominant particle size class at 1000 m at SKA was very fine sand (47%) followed by silt (26%).

This pattern within each site can be explained by two mechanisms. The first is that due to gravity, coarser particles tend to settle first whereas finer particles remain suspended in the air for longer and would settle further away from the source. According to the U.S. EPA, unpaved roads are responsible for 40% of atmospheric fugitive dust (EPA, 1997). Among these dust particles usually those smaller than 2.5 µm remain suspended in the air whereas the particles larger than 2.5 µm deposit much sooner, and the distance depends on the air movement and prevailing winds. Secondly is the role the MWAC sampler played at sampling various particle sizes. Though both Goosens and Offer (2000) and Waza et al. (2019) found that among all of the samplers they used in their respective studies the MWAC dust sampler was most efficient, Youssef et al. (2008) reported two main concerns regarding particle size and sampling height—both of which play an important role in the dust sampling efficiency of the sampler. They found that the MWAC sampler were not as efficient at sampling dust particle sizes less than 50 µm. It is, therefore, possible the MWAC sampler could have underestimated the distribution and amount of the smaller particle sizes, especially at distances further away from the road. Nonetheless, I show that the coarser particles deposited earlier and closer to the road at SKA, and to a lesser extent also at Wolwekraal, and in time this deposition will change the soil texture of adjacent areas. Conversely, finer particles are more likely to affect areas further away from the road.

Something that was also evident in this study was that dust loads at SKA and Wolwekraal were greater in the samplers at ground level compared to samplers at a height of 1.3 m, and the mean particle diameters were relatively large even at 1000 m away from the roads (including top and bottom samplers). The MWAC sampler is not as efficient at sampling dust at greater heights compared with near the ground (Youssef et al. 2008). However, the significant difference in mean dust loads and particle size between the surface and 1.3 m above the surface may not only be due to the MWAC sampler efficiency that varies between the height classes, but also because the bottom samplers possibly obtained dust from two different sources. The bottom samplers may have obtained dust from the road surface as well as soil particles transported by wind from the soil surface. Not only does this mean bottom samplers could have sampled greater dust loads, but also coarser particles due to the short distances surface-blown particles had to travel before they got deposited. This implies that shorter vegetation, as is the case in the Karoo, or young plants and saplings would receive higher dust loads and coarser particles, potentially physically damaging these vegetation types.

The majority of particles found in samplers at both sites were coarse. At Wolwekraal a markedly lower mean particle size (85.44 ± 46.49 µm) was found than at SKA (143.82 ± 59.13 µm). Mo et al. (2015) found that large particle size fractions (10–100 µm) accumulated more on the leaves of trees and shrubs compared to coarse PM (2.5–10 µm) and fine PM (0.2–2.5 µm). This suggests that plants at both SKA and Wolwekraal might be affected by artificially generated dust, as coarser particles are known to alter plant physiology and community structure (Brandt and Rhoades, 1972). However, both coarse and finer particles pose a threat to people or plants near unpaved roads, being capable of severe respiratory ailments and plant fitness reduction (Brown, 2009). Indeed, Hirano et al. (1991) determined the physical effects of dust on gas exchange and found that the smaller the particle size the greater the change in plant stomatal conductance during both dark and light periods. Coarse particles may block the stomata of leaves and reduce the transpiration and gas exchange of plants closer to the road whereas smaller particles can penetrate leaf stomata and internally impact plants by degrading the leaf chlorophyll of plants further from the road (Sett, 2017; Gunamani et al. 1991). Thus, the 0–20 m edge zone would be a particularly hazardous zone regarding long-term exposure to high loads of artificially generated dust, blocking leaf stomata off and influencing plant temperature, whereas areas further away should be impacted more from finer dust particles potentially penetrating stomata.

In contrary to expectations, Wolwekraal displayed an inconsistent pattern in mean dust load and particle size with distance from the road, whereas dust load decreased consistently with distance from the road at SKA. This could indicate that dust may have come from other sources including the road at Wolwekral, however the main factor believed to have played a role in the different pattern between the two sites is vegetation height. Rahul and Jain (2014) reported that various roadside tree species in their study were highly effective at capturing dust pollution emitted by vehicles. Most notably, Wolwekraal has a number of trees interspersed throughout the reserve, but which were completely absent at SKA (Figs. 2.1 and 2.10).

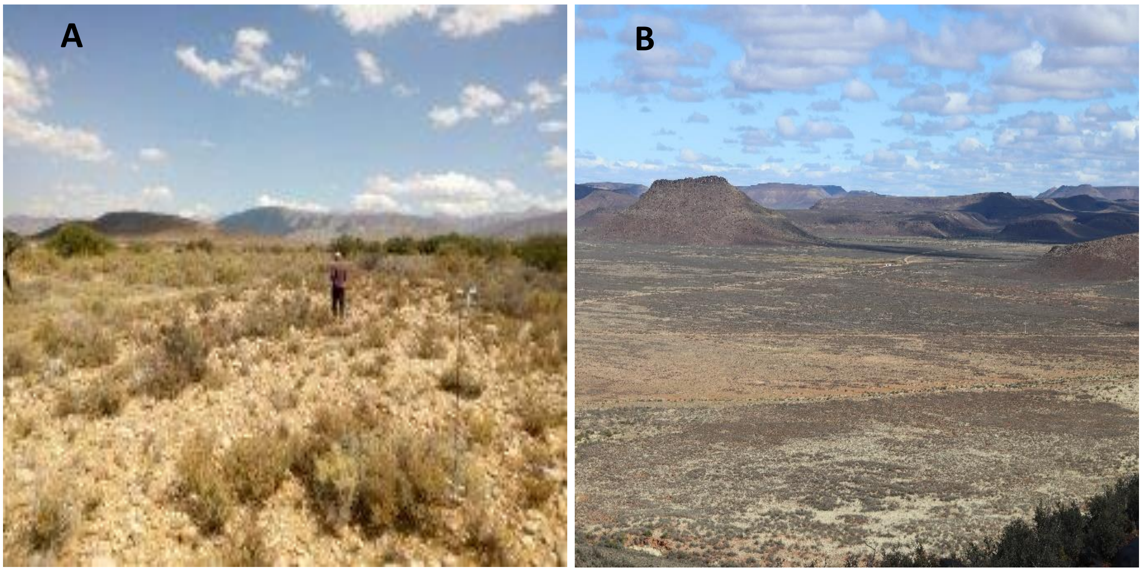

Figure 2.10. (A) Wolwekraal—low growing shrub and succulent vegetation but with tall trees interspersed. (B) SKA—low-growing shrubby vegetation, with lower numbers of succulents, and all relatively equal in height (see also Fig. 2.1). Photograph: Crous, 2018.

Another unexpected result comparing the two study sites was the fact that more dust was caught at SKA compared to Wolwekraal, even though the road next to Wolwekraal is a busy public road and the road at SKA a farm road with considerably lower traffic volumes. In contrast to the finding in this study, Nepali and Gyawali (2001) investigated road dust deposition amounts within three separate study sites. Two of the sites were situated at the crossing point of major highways, resulting in high dust deposition amounts due to high vehicle pressure compared to dust deposition in the other site that had much lower vehicle pressure. Although I believe the height of the vegetation played a role in the Wolwekraal case, the evidence at hand suggests soil texture did perhaps more so. The soil at SKA was sandier with more silt found at Wolwekraal, suggesting that the dust from the Wolwekraal road was also composed of finer particles in comparison to SKA, and thus the inefficiency of the MWAC at sampling smaller particle sizes could explain the large variation in dust loads captured between the two sites.

I recommended that the width of the inlet tube of the MWAC sampler should be increased at sites varying in vegetation structure, but only to a certain extent, as small insects or animals may infiltrate and block the tube. I found three of the 64 samples in Wolwekraal had spider infestations with webs spun that prevented dust entry into the inlet tube. A potential option would be to place the optimal width wire mesh into the inlet and outlet tube to prevent organisms from infiltrating but to still allow the appropriate particle sizes to pass through. Nevertheless, there is no one-size-fits-all approach to capturing dust spatially, and more care should be taken to account for the microtopography and soil texture of study sites. For relatively flat plains with evenly distributed low-growing vegetation, however, the method employed in this study seems appropriate to capture dust load and particle size characteristics from where to examine its effects on the environment.

To conclude, I provide clear evidence that the 0–20 m zone inwards from a road would be at risk of receiving higher dust loads. Of the two sites, SKA had significantly higher dust loads closer to the road while dust remained high, far from roads at Wolwekraal. This may have been an effect of finer particle sizes at Wolwekraal which also contributed to lower total dust loads even though the road was busier than at SKA. Based on the average dust loads found at the two sites compared to other similar studies that determined dust impacts on plant physiology, there is cause for concern in regards to plant health and fitness irrespective whether particles were predominantly fine or coarse. Dust loads found among similar studies varied considerably due to the different research environmental conditions and the sampling methods that were implemented, both of which depended on the research objectives of the particular study. Moreover, the dust generated from roads would alter the soil texture across a small scale, showering the nearby soils with particles with different particle sizes. Thus, unpaved roads may reduce ecosystem health near roads in the long run, and dust-reducing regulations would be key to mitigation. These regulations could include enforcing slower traffic speeds, or eventually covering/sealing these roads appropriately. This would imply that conservation agencies should consider the development of paved roads as one of the main priorities regarding dust-mitigation regulations.

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Chapter 3: Exploring the effects of dust from unpaved roads on plants in semi-arid Karoo landscapes

3.1. Introduction

Many regions are expected to become hotter and drier due to global climate change. In the semi-arid Karoo biomes of South Africa, plant cover is typically low, even though some regions like the Succulent Karoo remain rich in biodiversity. Increasing drought events would potentially further decrease plant diversity and cover in these Karoo drylands. Apart from climate change, or even poor rangeland management practices already impacting plant health in the Karoo, an aspect that has received little attention is dust pollution. Unpaved roads can be considered a major source of dust in arid landscapes. Many Karoo areas are historically underdeveloped, meaning most roads within these landscapes are unpaved, with vehicles sweeping up dust into the air and which are later deposited onto vegetation within the road's matrix. Thus, there is a need to explore the potential effects of dust generation from unpaved roads onto plants that are already growing under harsh environmental conditions, to better inform conservation planning and management.

A study conducted by Yoshida and Suzuki (1997) indicated that when dust gets deposited on the surfaces of leaves there is significant interference with gas exchange, due to particles covering or clogging the stomata in leaves. In the most severe cases, injury symptoms include necrosis and chlorosis of the leaves (Rahul and Jain, 2014). Leaf $\delta^{13}C$ values can provide a time-integrated indicator of photosynthetic activity in plants (Farquar et al. 1982). A higher $\delta^{13}C$ value is associated with lower gas exchange, and vice versa (Ehleringer and Cooper, 1988). Thus, as plant ecological strategy, light, nutrient and water availability would affect gas exchange (Ometto et al. 2006), dust deposition could also influence $\delta^{13}C$ in leaves.

Moraes et al. (2003) found a reduction in the net photosynthetic rate and other growth parameters, but increases in the concentrations of nitrogen and sulphur in *Tibouchina pulchra* within the Atlantic forest near an industrial complex in Cubatão, Brazil. Deposited dust can thus "fertilize" plants by increasing ammonium or nitrate availability (Xue et al. 2017). Nitrogen and its isotopes ($\delta^{15}N$) provide information on the nitrogen cycling within an ecosystem (Ometto et al. 2006). Garten et al. (2008) found that when nitrogen mineralization and N availability increased compared to the natural nitrogen supply gradient, the plant $\delta^{15}N$ values increased as well. In essence, $\delta^{15}N$ indicates variation in isotopic and chemical composition compared to, e.g., total soil nitrogen (Handley et al. 1999), as soil N may be accumulated via both ammonium and nitrates that differ in isotope values. $\delta^{15}N$ values thus also indicate the losses and assimilation of nitrogen by plants over time, creating variable fractionations (Handley et al. 1999). Furthermore, Grantz et al. (2003) reported that if particulate matter may affect fungi and bacteria in the rhizosphere, this could also influence nitrogen isotopes in plants. It is unknown what effect dust from gravel roads in the Karoo might have on the nitrogen dynamics of plant communities in the receiving landscape.

Air pollution has not only adverse physiological effects but has been known to alter leaf morphological traits such as the area, shape and colour (Swain, 2016). Specific leaf area (SLA), or the ratio of leaf area to leaf dry matter, is one of the best indicators of plant resource use, and the consensus is that SLA reflects a plant's expected return on resources that were previously used (Wilson et al. 1999; Wright et al. 2002). In particular, SLA can indicate the growth and abundance patterns of flora relative to nitrogen availability in semi-arid areas, indicating access to resources while other species cannot, and hence higher aridity tolerance (Wright et al. 2002; Liu et al. 2017). For example, a thicker leaf can prevent excessive water loss. High SLA leaves are productive and are best suited for resource-rich environments whereas, in resource-poor environments where the retention of resources is often the main priority, a low-SLA works best (Wilson et al. 1999). Thus, plants with low-SLA are geared to conserve their acquired resources in environmental conditions where resources are scarcer (Dreyer et al. 2003). They are predisposed to allocate resources towards areas other than the leaf, e.g., that which will aid in plant defense and survival (e.g. secondary metabolite production and root longevity) (Dreyer et al. 2003). Low SLA is a typical plant adaptation in order to survive drought cycles and high irradiances (Hoffman, 2005), e.g., such as the conditions plants experience in the Karoo biome. However, there are many exceptions where these generalizations does not hold for example, using SLA as a resource-stress indicator can sometimes be problematic as the leaf shape of a particular plant species significantly influences SLA, because the shape influence leaf density, a pivotal factor of SLA (Wilson et al. 1999).Sarma et al. (2017) observed a decrease in SLA of three shrub species next to the National Highway 37 (NH-37) compared to the control (vehicle-free zone). They found a strong negative correlation between dust loads and SLA. In some plants, leaf length, width and petioles may also be affected by high dust load deposition (Miller et al. 1973). Similarly, Verma and Singh (2006) found physical changes in the leaf area and leaf surface characteristics of both *Ficus religiosa* and *Thevetia neriifolia* in vehicle-polluted areas.

The toxic metals that traffic releases into the environment, particularly those near the vicinity of roads, is cause for further concern, possibly impacting plant physiology and ultimately survival, as well as that of biota that consumes affected plants (Kim et al. 2015). Trace metals are a real threat toroadside plants due to their long residual times and non-degradability (Yan et al. 2018). Dust from roads contaminated by trace metals originates from human activities, such as waste disposal, energy production, fuel combustion, and vehicle exhaust fumes (Gawade, 2016). Dust can, therefore, affect plants indirectly via changing the surrounding soil chemistry and some metal ions can enter the roots directly when dissolved in water (Farmer, 1991).

Road dust that contains toxic metals or calcium hydroxide can infiltrate the leaf tissue if particles are small enough, which can lead to cell plasmolysis and the eventual death of plants (Gheorghe and Ion, 2011). Heavy metals such as Pb and Cu have been known to indirectly interfere with electron transport processes. Lead usually comes from fuel combustion and vehicle exhaust when vehicles use leaded petrol, however, Kurkjian et al. (2003) suggest that trace amounts of lead can still be found in unleaded gasoline, whereas spillage of lubricants in vehicles and metallic corrosion can produce copper. Trace metals such as Zn is essential for growth and development in plants because they play an important role in protein, auxin and enzyme synthesis. Zinc is a vulcanization agent of tyres and can occur in high concentrations in road dust if tyre abrasion in vehicles occurs (Gawade, 2016). A decrease in plant performance and biochemical modifications can be observed when there are alterations in plant Zn concentrations (Rai, 2016). Chromium in the form of organic compounds can be essential to plants, but above critical levels it can result in the reduction of root and shoot growth rates (Faisel and Hasain, 2006). Nickel is another metal that is highly associated with traffic and is essential for plant growth and nitrogen metabolism, but in toxic concentrations it can affect photosynthesis by interrupting electron transport from pheophytin (Bhalerao et al. 2015). Thus, when a plant is already living under harsh environmentalconditions, e.g., in arid regions, dust pollution can render it more susceptible to dieback and decline by modifying the host's physiology (Rahul and Jain, 2014).

In Chapter 2, I showed that the source material from unpaved roads would impact the characteristics of the vehicle-generated dust flowing into the vegetated matrix; for example, different roads may have varying particle sizes and produce varying dust loads. Furthermore, the ability of a plant to retain and remove dust depends on the complexity of the stem structure but also on leaf traits that absorb particles from the atmosphere more easily such as leaf textures which include hairs, moisture and grease or wax on the leaves (Zhang et al. 2015). Thus, plant communities next to unpaved roads might be impacted differently by dust in space and time, suggesting no one-size-fits-all approach to predict the impacts of dust on plants. In this chapter, I investigate 1) whether road dust has the potential to physiologically impact plants by altering their natural carbon and nitrogen intake and assimilation, which could lead to a decrease in photosynthetic performance of roadside plants; 2) if there will be any impact on SLA, as a morphological plant-stress indicator, due to increasing dust loads next to roads; and 3) if there are traces of pre-selected heavy and trace metals associated with road dust in and on leaves of two typical Karoo plant species found next to and further in-field of an unpaved road. I hypothesize that distance from the road will have a significant impact on the resource-use traits of plant species and, similarly, that plants closest to the road will have a higher concentration of heavy metals at the distance closest to the road, where dust loads are higher (Chapter 2). This functional approach to understanding the effects of dust pollution on plants would aid conservationists to better understand the nature of the environmental impacts facing arid and dust-prone areas of South Africa today, and also in the future.

3.2. Materials and methods

3.2.1. Short description of plant sampling procedure and leaf characteristics

The study sites and distance-transect layout follow Chapter 2. In short, two species in each site were sampled, two shrubs (*Pteronia glauca* and *Rhigozum trichotomum*) in SKA and one shrub (*Pteronia pallens*) and a succulent (*Ruschia spinosa*) in Wolwekraal Nature Reserve. Then, within each of the four distance categories (D1 = 0–20 m; D2 = 20–100 m; D3 = 100–400 m; and D4 = 400–1000 m), at least 100 leaves of each of eight plants (four plants per species) were sampled next to each of the dust samplers, without stripping off more than 25% of the leaf cover. Branches from all sides were sampled and the height and width of each plant were measured using a tape. A total of 32 individuals (16 per species) were sampled per site. Table 3.1 gives a short description of the leaf characteristics of these four species.

Table 3.1. Summary of the leaf characteristics of *Pteronia glauca*, *Pteronia pallens*, *Rhigozum trichotomum* and *Ruschia spinosa* that may play a role in the amount of dust that remain on leaves after deposition.

Plant species	**Leaf characteristics**	**Dust capturing and retainment potential**
Pteronia glauca	Triangular shaped, small leaves (<5 mm). Branches that carry leaves are usually curved towards the ground surface	**Low** (Small leaves and leaves curved to the ground lose dust more easily)
Pteronia pallens	Smooth, fleshy leaves that occur in tufts. Leaves are narrow and long and are blunt-ended and thread-like	**Low** (Dust is easily removed on smooth and narrow leaves)
Rhigozum trichotomum	Hairy, tiny leaves that grow in whorls of three. The leaves have entire, waxy margins and they fold inwards along the central axis	**High** (Leaves are hairy, waxy and leaf folding further enhances dust accumulation)
Ruschia spinosa	Tick, fleshy short and squat leaves. The lower leaf surface bulges to hold and support succulence in the leaf whereas the upper surface is nearly flat	**Medium** (Leaves are fleshy and usually have cuticle, but are also short and squat)

3.2.2. Carbon and nitrogen isotopes

The same 50 leaves used to determine SLA samples were ground into finer particles by hand in a mortar and pestle, weighed to be between 0.5 to 0.9 mg, and placed into tin cups that were sent to iThemba Labs (University of Witwatersrand, Johannesburg, a facility of National Research Foundation) for carbon and nitrogen isotope analyses. Analyses were done on a Flash HT Plus elemental analyser coupled to a Delta V Advantage isotope ratio mass spectrometer by a ConFloIV interface (all equipment supplied by ThermoFisher, Bremen, Germany). Carbon and nitrogen isotope values were corrected against an in-house standard (Merck Gel) and a Urea Working Standard (IVA Analysentechnik e.K., Meerbusch, Germany). Laboratory standards were run after every 24 samples, the maximum sample number the Flash HT Plus elemental analyser could analyse at a time.

3.2.3. Specific leaf area (SLA)

All plant leaf samples were dried at 40ºC for three days until a constant weight was achieved. The weight of 50 leaves per sample from each plant species was measured using a scale (A&D, HR100AZ) and the areas of the three shrub species were determined by Image J software. The shape of the succulent species leaves looked spherical when observed from the naked eye; thus, the leaf surface area was calculated by using the formula $4\pi r^2$ in which the radius was determined by Image J software. The SLA for all of the 50 leaves per sample was calculated by dividing the final determined area with its respective leaf dry mass. The SLA of each plant species was thus calculated as $cm^2\ g^{-1}$.

3.2.4. Inductively coupled plasma mass spectrometry (ICP-MS)

Due to cost constraints, only the remaining leaves from plant species at SKA were sent for ICP-MS which analysed for two heavy metals: Cu and Pb and three trace metals Cr, Zn and Ni. Leaves were oven-dried at 40°C for three to four days, weighed to 0.5 g using a scale (A&D, HR100AZ) and turned into a powder using an agate mill for the digestion process. In order to keep the remaining dust on the leaves, none of the leaves was rinsed off with water. This was also to ensure that high concentrations of metals were found so that concentrations did not fall below the detection limits of the ICP-MS. All digestions used Merck Suprapure HNO_3 within Teflons to avoid contamination of samples. Table 3.1 contains information regarding the leaf characteristics of the four plant species observed at the two study sites.

3.2.5. Statistical analyses

All data were tested for normality by means of a Shapiro–Wilk test. The Kruskal–Wallis multiple comparison of mean ranks was used to determine significant differences of $\delta^{13}C$ at Wolwekraal and $\delta^{15}N$ at SKA between distances. A factorial ANOVA was used to determine significant differences in SLA of leaves from species between distances at Wolwekraal and SKA. Similarly, the factorial ANOVA was used to determine significant differences of $\delta^{15}N$ at Wolwekraal and $\delta^{13}C$ at SKA between distances. A Kruskal–Wallis multiple comparison of mean ranks was conducted to determine significant differences in the deposition of metal ions between distances deposited on and in the leaves of *Pteronia glauca* and *Rhigozum trichotomum* at SKA. Furthermore, a Spearman's rank correlation was conducted to determine the strength of the relationship of SLA with $\delta^{15}N$, $\delta^{13}C$ and metal ions respectively.

Finally, since there was a full dataset for SKA, I wanted to disentangle the unique and combined (co-varying) effects that distance from the unpaved road and plant species may have on the measured leaf metal concentrations and physiological traits (SLA, C and N isotopes). I did so by using a redundancy analysis (RDA) CANOCO 5 (Microcomputer Power, Ithaca; Ter Braak and Šmilauer, 2012). The forward selection procedure in the CANOCO software, using a permutation test with 9999 permutations, was used to identify the strongest categories that could help explain the variation in leaf metal concentrations and physiological traits. Variance partitioning (testing conditional effects using 9999 permutations) was then performed to determine the unique and combined contribution of each significant variable group ("Distance" and "Species") in explaining concentration and trait variation (Borcard et al. 1992). Covariates that were linearly dependent were ignored in these analyses (in this case the distance *D1* and the species *R. trichotomum*) but included in the final ordination scheme (Lepš and Šmilauer 2003). In all cases we used the adjusted R^2 variation to account for sample size and the number of exploratory variables included (Peres-Neto et al. 2006). Response data were standardized.

3.3. Results

3.3.1. Leaf Carbon and Nitrogen Isotopes for Species at Wolwekraal and SKA

Both leaf $\delta^{15}N$ and $\delta^{13}C$ were calculated for each species at Wolwekraal and SKA. Plant leaf $\delta^{15}N$ was determined to give an idea of the rate of nitrogen cycling and nitrogen status of each plant,whereas $\delta^{13}C$ provide an estimate of a plants' water use efficiency. At Wolwekraal *R.*

spinosa ($\delta^{13}C$ = -23.18 ± 1.05) had a greater mean $\delta^{13}C$ than *P. pallens* ($\delta^{13}C$ = -26.08 ± 0.06) (Fig. 3.1A). *R. spinosa* ($\delta^{15}N$ = 7.77 ± 1.14) also had a greater mean $\delta^{15}N$ than what was found within *P. pallens* ($\delta^{15}N$ = 4.42 ± 0.73) (Fig. 3.1B). At SKA *R. trichotomum* ($\delta^{13}C$ = -27.08 ± 1.06) had a higher mean $\delta^{13}C$ compared to *P. glauca* ($\delta^{13}C$ = -28.35 ± 1.11) (Fig. 3.1C). The mean $\delta^{15}N$, however, was lower in *R. trichotomum* ($\delta^{15}N$ = 3.94 ± 1.13) than in *P. glauca* ($\delta^{15}N$ = 4.87 ± 1.33) (Fig. 3.1D). The only significant difference ($p = 0.006$) was found at SKA between $\delta^{15}N$ at D1 and $\delta^{15}N$ at D4 of *P. glauca* (Fig. 3.1D).

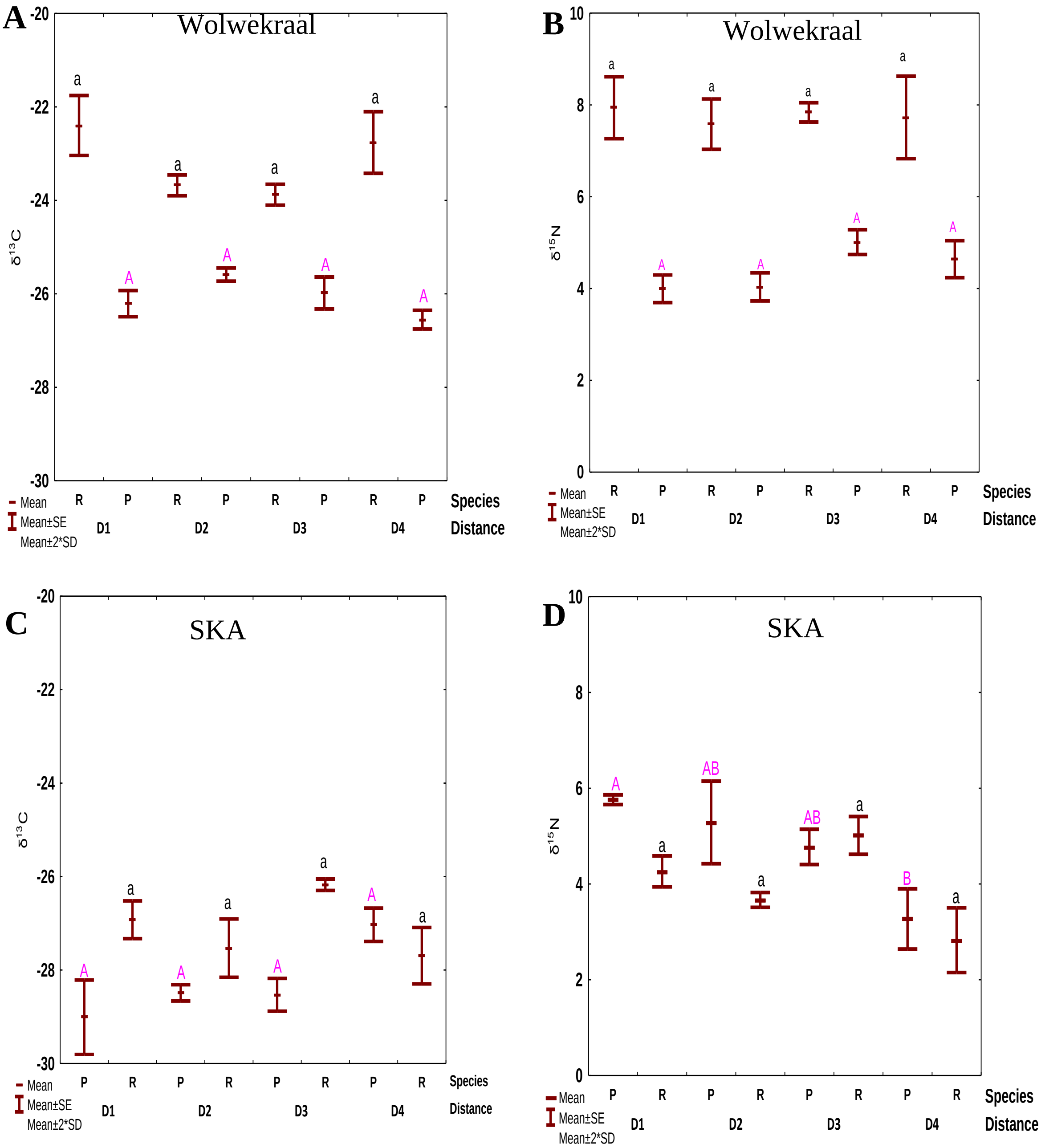

Figure 3.1. (A) The mean $\delta^{13}C$ values for plants at Wolwekraal; (B) The mean $\delta^{15}N$ values for plants at Wolwekraal; (C) The mean $\delta^{13}C$ values for plants at SKA and (D) The mean $\delta^{15}N$ values for plants at SKA. For graph A, small letters show statistical significance of *R. spinosa's* mean $\delta^{13}C$ among distance, whereas capital letters show statistical significance of *P. pallens's* mean $\delta^{13}C$ among distances; for graph B small letters show statistical significance of *R. spinosa's* mean $\delta^{13}C$ among distances, whereas capital letters show statistical significance of *P. pallens's* mean $\delta^{13}C$ among distances; for graph C small letters show statistical significance of *R. trichotomum's* mean $\delta^{13}C$ among distances, whereas capital letters show statistical significance of *P. pallens's* mean $\delta^{13}C$ among distances; for graph D small letters show statistical significance of *R. trichotomum's* mean $\delta^{15}N$ among distances, whereas capital letters show statistical significance of *P. pallens's* mean $\delta^{15}N$ between distances. Different letters indicate significant difference at α=0.05; (A, B) R= R. *spinosa* and P= *P. pallens*, (C, D) R= *R. trichotomum* and P= *P. glauca*; D1= 0-20 m, D2= 21-100, D3= 101-400 m and D4= 401-1000 m.

3.3.2. Specific Leaf Area (SLA) of Species at Wolwekraal and SKA

The mean SLA of *P. pallens* (28.9 cm^2 g^{-1} ± 4.94) and *R. spinosa* (35.5 cm^2 g^{-1} ± 3.65) at Wolwekraal (Fig 3.2A) and *P. glauca* (44.0 cm^2 g^{-1} ± 5.00) and *R. trichotomum* (57.0 cm^2 g^{-1} ± 8.62) at SKA (Fig 3.2B) was measured and was higher for the two species at SKA than at Wolwekraal, but there were no differences between species within a site.

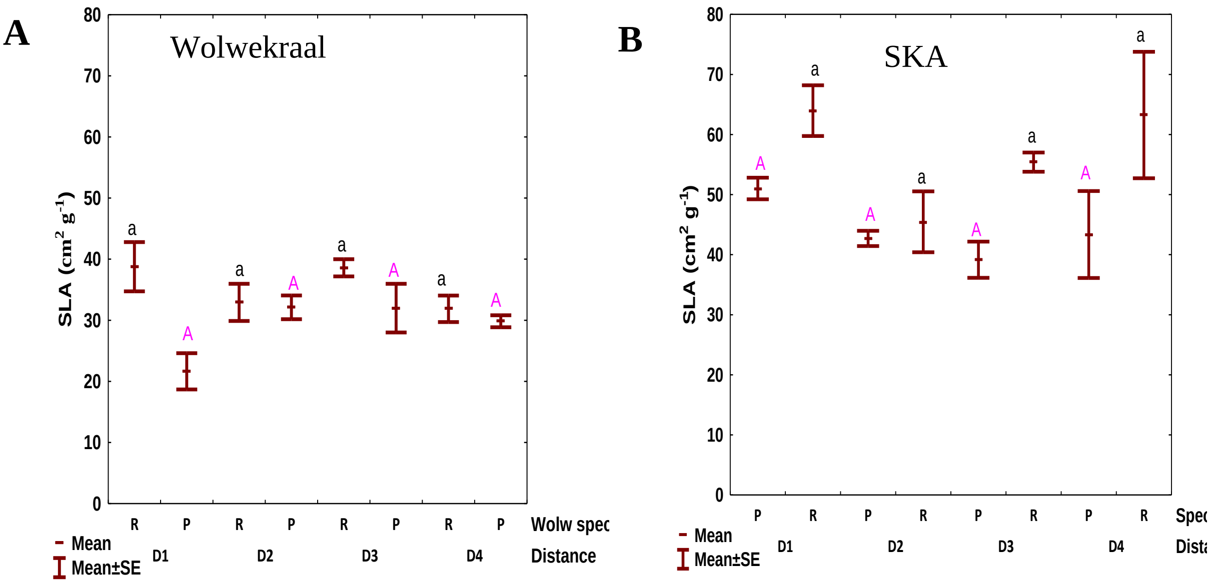

Figure 3.2: (A) The mean SLA of plant leaves at Wolwekraal and; (B) The mean SLA of plant leaves at SKA; (A) Small letters show statistical significance of *R. spinosa*'s mean SLA among distances, whereas capital letters show statistical significance of *P. pallens*'s mean SLA among distances; (B) Small letters show statistical significance of *R. trichotomum* 's mean SLA among distances, whereas capital letters show statistical significance of *P. glauca's* mean SLA among distances. Different letters indicate significant difference at α=0.05; (A) R= *R. spinosa* and P= *P. pallens*; (B) R= *R. trichotomum* and P= *P. glauca*; D1= 0-20 m, D2= 21-100, D3= 101-400 m and D4= 401-1000 m.

None of the species mean SLA displayed any significant differences between distances at Wolwekraal and SKA. However, all of the species displayed an inconsistent pattern of mean SLA along the distance gradient. At Wolwekraal the mean SLA of *R. spinosa* was highest at D1 (38.77 cm^2 g^{-1}) and lowest at D4 (31.88 cm^2 g^{-1}) and highest at D2 (32.12 cm^2 g^{-1}) and lowest at D1 (21.65 cm^2 g^{-1}) for *P. pallens* (Fig. 3.2A). At SKA the mean SLA of *R. trichotomum* was highest at D1 (63.98 cm^2 g^{-1}) and lowest at D2 (45.47 cm^2 g^{-1}) and highest at D1 (51 cm^2 g^{-1}) and lowest at D3 (39.16 cm^2 g^{-1}) for *P. glauca* (Fig 3.2B). All of the species had the highest mean SLA at D1 among all of the distances with the exception of *P. pallens* at Wolwekraal.

3.3.3. Leaf Heavy and Trace Metal Concentration

ICP-MS was used to determine the amounts of metal ions, including Cu, Zn, Cr, Ni, and Pb, within and on the surface using 500 mg of the leaf material from *P. glauca* and *R. trichotomum* at SKA only. High concentrations of all metal ions were found within and on the surfaces of the leaves from both species. In order from most to least, the average amounts of metal ions that were found on both species was Zn (18251 µg kg^{-1} ± 14298) > Cu (6301 µg kg^{-1} ± 5693) > Cr (1681 µg kg^{-1} ± 943.17) > Ni (827 µg kg^{-1} ± 335.62) > Pb (559 µg kg^{-1} ± 338.55) (Fig. 3.3A–E). There were higher mean amounts of Cu (9467 µg kg^{-1}) and Zn (28 191 µg kg^{-1}) (Fig 3.3A,B) found within and on the leaf surfaces of *P. glauca* compared to *R. trichotomum*, whereas *R. trichotomum* had higher mean amounts of Cr (2470 µg kg^{-1}), Ni (1067 µg kg^{-1}) and Pb (841 µg kg^{-1}) (Fig. 3.3C–E) within and on the leaf surfaces compared to *P. glauca* (includes means combined from all distances).

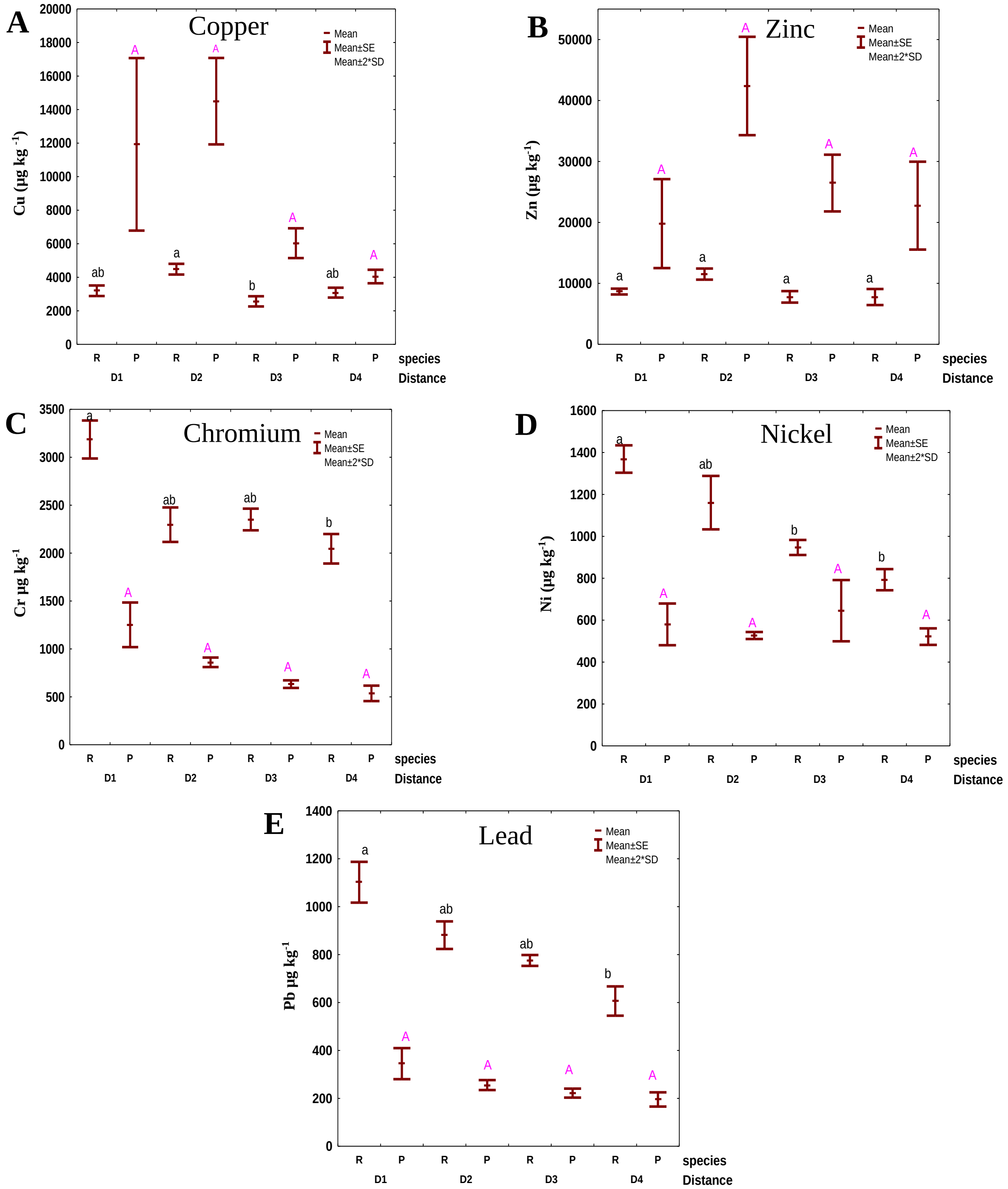

Figure 3.3. (A) Mean concentration of Cu within leaves and on leaf surfaces; (B) Mean concentration of Zn within leaves and on leaf surfaces; (C) Mean concentration of Cr within leaves and on leaf surfaces; (D) Mean concentration of Ni within leaves and on leaf surfaces; (E) Mean concentration of Pb within leaves and on leaf surfaces; (A to E) Small letters show statistical significance of *R. trichotomum's* mean Cu, Zn, Cr, Ni and Pb among distances, whereas capital letters show statistical significance of *P. glauca's* mean Cu, Zn, Cr, Ni and Pb among distances and different letters indicate significant difference at α=0.05; R= *R. trichotomum* and P= *P. glauca*; D1= 0-20 m, D2= 21-100, D3= 101-400 m and D4= 401-1000 m.

P. glauca had more than double the concentration of Cr at D1 (1252 µg kg^{-1}) compared to D4 (537 µg kg^{-1}), and almost double the concentration was found for *R. trichotomum* at D1 (2219 µg kg^{-1}) than what was found at D4 (1399 µg kg^{-1}). The concentrations of Cu were more than double for *P. glauca* at D1 (11929 µg kg^{-1}) compared to D4 (4047 µg kg^{-1}) and similarly for *R. trichotomum* at D1 (7563 µg kg^{-1}) compared to D4 (3497 µg kg^{-1}). For both species a similar pattern for Zn was found where its concentration increased from D1 to D2 (Zn concentrations were highest at D2 for both species), however concentrations consistently decreased from D2 right through to D4. Both Pb and Cr were the only metals that consistently decreased with distance from the road for both plant species.

3.3.4. Multivariate Variance Partitioning for the SKA Dataset

Heavy metal concentration and leaf C and N isotope levels were significantly influenced by both the distance away from the unpaved road as well as species (all forwardly selected variables had a $P < 0.01$ after 9999 permutations; Table 3.2). In total, these two groups of variables explained 52.3% of all the observed variation. However, after the conditional partitioning of the variance, it was clear that species type, fraction *b* in this case, was stronger in influencing leaf elemental traits than the distance from the unpaved road, fraction *a* (Fig. 3.4). Specifically, fraction *b* explained 47.4% of the variation ($F = 28.8$, $P < 0.001$), and fraction *a* only explained 11.8% ($F = 3.5$, $P < 0.001$). Nevertheless, although fraction *a* explained a smaller proportion of the variation compared to fraction *b*, both groups are important to determine leaf metal and isotopes abundance in this Karoo landscape (tested fraction *a*+*b*+*c*: $F = 9.8$, $P < 0.001$). Furthermore, the negative value of the shared fraction *c* (-5.2%) confirms that distance and species together had a significant yet markedly opposite effect on leaf metal and isotope abundance (see Peres-Neto et al. 2006). This means, for example, that some metals were higher in a particular plant species, but that distance from the road significantly decreased this association. In this case, higher metal exposure at D1, due to higher dust loads in D1 (see Chapter 2), was more associated with *R. trichotomum* than *P. glauca*, but these concentrations dwindled as one moved away from the unpaved road, particularly at D4 but lesser so at D2 and D3 (as they were not significant explanatory variables). This suggests that D1, the 0–20 m corridor next to these unpaved roads, may be particularly impacted by heavy metal pollution and associated leaf elemental trait changes in some plant species.

Table 3.2. Interactive-forward-selection after performing an RDA analysis, indicating the most significant categories of variables within “Distance” (n = 4) and “Species” (n = 2) on leaf metal and isotope concentrations.

Name	**pseudo-F**	**P-value**
Pteronia glauca	22.9	0.0001
Rhigozum trichotomum	22.9	0.0001
Distance D4	4.5	0.0003
Distance D1	3.3	0.005

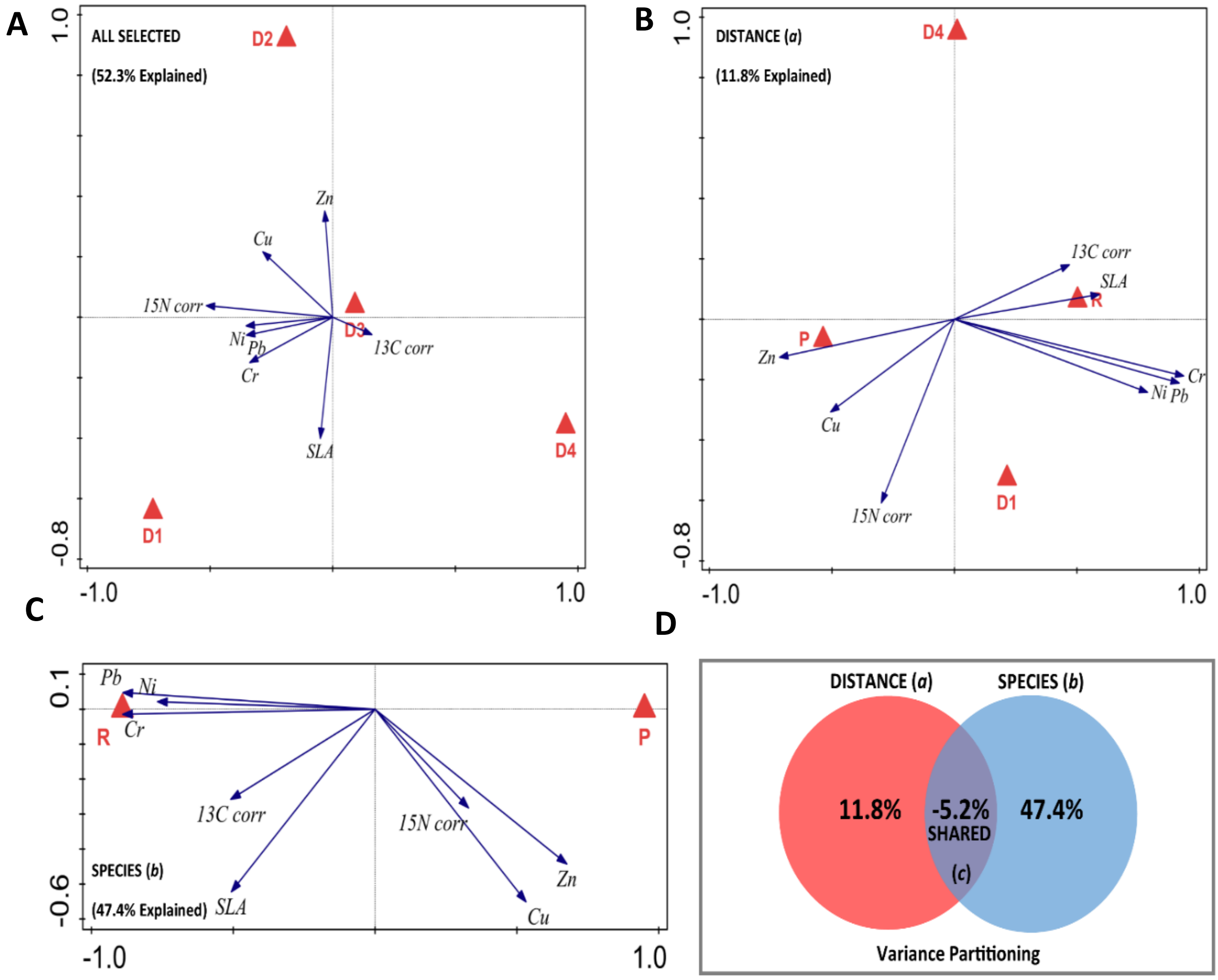

Figure 3.4. (A) Redundancy analysis (RDA), showing how distance from an unpaved road and plant species significantly influenced leaf metal, SLA and C and N isotope abundance at SKA; (B) Redundancy analysis (RDA), showing how distance only from an unpaved road significantly influenced leaf metal, SLA and C and N isotope abundance at SKA; (C) Redundancy analysis (RDA), showing how plant species only significantly influenced leaf metal, SLA and C and N isotope abundance at SKA; (D) Variance partitioning performed to determine the unique and combined contribution of each significant variable group ("Distance" and "Species") in explaining metal concentration and leaf trait variation (SLA and C and N isotope abundance) at SKA. The total adjusted variation was 52.3%, and all forwardly selected variables had a P-value < 0.01 after 9999 permutations. Tested fraction *a*+*b*+*c*: F = 9.8, P < 0.001; Tested fraction *a*: F = 3.5, P < 0.001; Tested fraction *b*: F = 28.8, P < 0.001. Note the negative value of shared fraction *c*, confirming the strong yet opposite effects that groups *a* and *b* had on the response variables (Peres-Neto et al. 2006). D1 = 0–20 m and D4 = 400–1000 m; P = *P. glauca* and R = *R. trichotomum*.

3.4. Discussion

At both Wolwekraal and SKA, $\delta^{13}C$ and $\delta^{15}N$ did not differ significantly among distances for each respective plant species, with the exception of *P. glauca* where $\delta^{15}N$ differed significantly between the closest (0-20 m) and furthest (401-1000 m) distance from the road. Similarly, SLA did not differ significantly among distances for any of the plant species at both sites. *P. pallens* had the lowest mean SLA (28.9 cm2 g-1 ± 4.94) and *R. trichotomum* (57.0 cm2 g-1 ± 8.62) had the highest. At SKA, *P. glauca* had no significant differences in the mean concentrations of any of the five metals among distances, whereas *R. trichotomum* only had significant differences in the concentrations of metals- Cr, Ni, and lead between the distance closest (0-20 m) to road and distances further away from the road.

The higher the $\delta^{13}C$ values the more water-use efficient a plant is, but $\delta^{13}C$ can also be affected by water availability and distribution across the landscape. However, based on the results of the redundancy analysis it is clear that the effect of distance on $\delta^{13}C$ was lower in magnitude compared to effect of the individual plant species itself. Among all four species at both sites *P. glauca* had the lowest $\delta^{13}C$ values (-29.01 ± 1.59) at 0–20 m compared to all of the other distance categories away from the road. *P. glauca* at SKA was the only species that had a consistent increase in $\delta^{13}C$ values with distance from the road, whereas the other three species all displayed an inconsistent pattern in $\delta^{13}C$ values.). In Chapter 2 we found that at SKA larger mean particle sizes were found than at Wolwekraal, and particles at SKA were coarser at 0-20 m compared to distances further way from the road. Dust has the potential to penetrate the stomata, rupturing them, and larger particles may prevent complete stomatal closure, leading to drought stress in plants, which is especially negative in arid regions (Van Heerden et al. 2007). The larger particles that *P. glauca* at 0-20 m at SKA received, may explain why its $\delta^{13}C$ values were the lowest at the distance closest to the road. For example; Sharifi et al. (1997) determined the difference in WUE of three species *Larrea tridentate*, *Hymenoclea salsola* and *Atriplex canescens* that were dusted and non-dusted (control). They found that WUE of dusted *H. salsola* and *L. tridentate* were significantly lower compared to non-dusted plants, however WUE remained unchanged for *A. canescens*. The authors believed that a decrease in plant WUE may have been the result of dust particles that prevented the stomata from closing, which increased transpiration and water loss (Rahul and Jain, 2014). Plant WUE may also be influenced by an increase in leaf temperature. Road dust on leaves absorbs more radiation, particularly energy from wavelengths over 700 nm causing an increase in transpiration and water loss (Eller, 1970). Thus, the higher dust laods at SKA could have increased the dust's potential to absorb more radiation. This in combination with the impacts of larger particles found at SKA, could explain why mean $\delta^{13}C$ for the two species at SKA was higher than the two species at Wolwekraal. In turn, some plant species evolved mechanisms to adapt and cope with water loss and perhaps also dust pollution. For example, *R. spinosa* at Wolwekraal, a CAM plant that had the highest $\delta^{13}C$ of all species in line with this photosynthetic pathway, which remained high even directly next to the road.

Van Heerden et al. (2007) measured various physiological and morphological parameters of plants at increasing distances away from a limestone quarry. There was a decrease in leaf area of plants found closest to the source compared to distances further away; however, WUE was similar among all of the distances. The plants closest to the source were most under stress due to the intensive deposition of dust and may, therefore, adapt by synthesizing more wax on their leaves to decrease water evaporation and be more drought resistant (Saneoka and Ogata, 1987).

Similar results were found for the values of $\delta^{15}N$ in that not much difference was found in the $\delta^{15}N$ among the four species with distance. Wolwekraal especially displayed little differences in $\delta^{15}N$ between distances, while at SKA the general trend was that the $\delta^{15}N$ at the furthest distance was lower, but the plants closer to the road and one another had similar values. Specifically, again only *Pteronia glauca* had $\delta^{15}N$ values significantly higher at 0–20 m (5.76 ± 0.20) compared to 400-1000 m (3.27 ± 1.09). It therefore seems that *P. glauca* individuals closer to the road are accumulating more of the heavier nitrogen isotopes than plants at distances further away from the road (Ometto et al. 2006; Gurmesa et al. 2017). However, this may also be due to higher soil fertility at 0-20 m or nitrogen received from other sources. This is similar for *R. trichotomum*, where $\delta^{15}N$ was highest at 0–20 m compared to the other distances although there were no significant differences. Sarma et al. (2017) suggested that when the photosynthetic capacity, growth and development of roadside plants are impacted by high dust loads, plants are unable to take up nitrogen when they are exposed to such detrimental conditions. In the current study, however, with the exception of $\delta^{15}N$ in *P. glauca* at SKA, the physiological indicators included ($\delta^{15}N$, $\delta^{13}C$, and SLA) suggest only minor water and nitrogen-use impacts on plants due to dust deposition.

However, in contrast to the findings of Sarma et al. (2017), a few studies found that depending on the chemical composition, dust pollution may actually be a nitrogen source to plants (Moraes et al. 2003; Xue et al. 2017). What was interesting was that based on redundancy analysis $\delta^{15}N$ increased at D1(0-20 m) relative to D4 (400-1000 m) at SKA which may support findings by Moraes et al. (2003) and Xue et al. (2017), suggesting that transported dust has the potential to provide vegetation with varying nitrogen sources. As was expected; the succulent *R. spinosa* seemed to be the species that was functioning the most dissimilar under similar environmental conditions, hence the higher average $\delta^{15}N$ (7.77 ± 1.14) and $\delta^{13}C$ (-23.18 ± 1.05).

Usually $\delta^{15}N$ will be higher in plants living in more arid conditions, this explain why $\delta^{15}N$ values here are higher than $\delta^{15}N$ that are usually found in forests, for example. Plant nutrients, which include metals, generally have a strong relationship with SLA but water availability can influence this relationship (Hoffman et al. 2005). SLA gives an idea of how plants are adapted to their environment and are linked to their photosynthetic capacity. It can be influenced by water uptake, light availability and nutrient availability (Karavin, 2013). We also found that there was a moderately weak negative correlation between SLA and the metals with the highest concentrations Cu and Zn, whereas SLA shared a moderately weak/strong positive correlation with Cr, Ni and Pb, and all of the correlations were significant ($p > 0.05$). The fact that only negative correlations of SLA were with copper and zinc, which was in highest concentrations in on plant leaves could indicate that they have exceeded the toxic limitations of the plants, since the higher concentrations of these metals resulted in a reduction of SLA.

Due to the weak to moderately weak correlations SLA had with $\delta^{13}C$ and $\delta^{15}N$ at SKA (SLA and $\delta^{13}C$ ($r = 0.28$), SLA and $\delta^{15}N$ ($r = 0.06$)) and at Wolwekraal (SLA of *P. pallens* and $\delta^{13}C$ ($r = 0.12$), SLA of *P. pallens* and $\delta^{15}N$ ($r = 0.25$), SLA of *R. trichotomum* and $\delta^{13}C$ ($r= 0.25$), SLA of *P. pallens* and $\delta^{15}N$ ($r = 0.25$)), it is safe to assume that SLA cannot be used as a predictor of these physiological traits. However physiological traits such as water use efficiency and nitrogen cycling aren't the only variables that could have influenced SLA. Variables not measured in the present study such as leaf life span, leaf senescence, shading and plant age are all variables likely to correlate with SLA as other researchers have shown them to significantly influence SLA (Jacobsen et al. 2008; Karavin, 2013; Liu et al. 2016). Non-convergence in SLA among species has been shown for shrubs in the arid lands of southern California (Jacobsen et al. 2008), supporting the view that there will be differential responses to dust pollution within Karoo plant communities.

There was a clear trend that heavy metal concentrations were affected by both distance from the road and the specific plant species examined. The published research on roadside heavy/trace metal contamination was mostly conducted in developed countries that have a long history of extensive use of leaded gasoline and industrialization. Very few studies attempted to evaluate heavy/trace metal concentrations on and in plants along a distance gradient from roads in developing countries, and even fewer were carried out in arid environments in the vicinity of unpaved roads. At SKA the highest metal concentration was for Zn (18251 µg kg^{-1} ± 14298), whereas Pb (559 µg kg^{-1} ± 338.55) was found in the lowest mean concentration. A few studies report similar results in which out of all the evaluated metals Zn had the highest mean concentration; however, the mean Zn and Pb concentration found in and on plants in this study were much higher compared to what was found in plant leaves in most other similar studies. Jardat and Momani (1999) found highest Zn (0.060 µg kg^{-1}) concentrations and lowest Pb concentrations (0.005 µg kg^{-1}) in plants next to the main road in Aman city. In contrast to the results of this study and the study conducted by Jardat and Momani (1999), Tanee and Albert (2013) measured Zn and Pb in plants next to three highways in the Rivers state of Nigeria (Refinery Road, Akpajo-Onne Junction and Aleto-Eleme By-Pass Road) with relatively heavy traffic. Within *Panicum maximum* and *Centrosema pubescens*, Zn values ranged from 43280–84520 µg kg^{-1} whereas Pb concentration was 136680-278400 µg kg^{-1}. The Zn and Pb concentrations found by Tanee and Albert (2013) in plant leaves were considerably higher than the concentrations found by Jardat and Momani (1999) and the current study.

The difference in the results obtained between the three studies indicates the importance of the different environments that the plants grew in. The three Nigerian highways discussed by Tanee and Albert (2013) had more than 2000 vehicles that drove past on a daily basis, coupled with two petroleum refining plants and other refined oil storage companies within the Eleme local government area, which could also explain why concentrations of Pb were higher than concentrations of Zn in plants. In the other two studies where Pb concentration was much lower, traffic rates were not nearly as high and sites were situated within rural areas. It should also be noted that within this study, metal concentrations were obtained from combined measurement of metals within plant leaves and within dust that deposited on plant leaf surfaces, whereas in most similar studies, including the other two studies, metal concentrations only within plant leaves were measured. Also, the other two studies were conducted near paved roads whereas in this study unpaved roads served as a major source of metal concentrations found on and within plant leaves. Thus, which types of metals and their concentrations observed will depend on environmental and anthropogenic factors, the type and amount of pollution sources, and very importantly the plant species.

Due to the persistent and accumulative characteristics of heavy metals in soils, the effect of Pb in the soils from past events could have been expected. However, it is clear that roadside management of these heavy metals must be accounted for. Among the five metals Cr, Pb and Ni are carcinogenic (Kim et al. 2015; Wang et al. 2017). Organisms exposed to high concentrations of these metals may undergo disruption of repair damage processes, enzyme activities regarding oxidative damage and tumour suppressor gene expression (Kim et al. 2015). The fact that Cr, Pb, and Ni increased along vegetation on the road verge is cause for concern, especially where food products are produced next to unpaved roads using evergreen and long-term species such as trees, as well as for people living close to the road. Indeed, heavy metals are indicators of the long-term anthropogenic pollution an area receives. These metals may not be sufficient by themselves to induce cancer however they may increase the risk of carcinogenic events involved in DNA damage/repair when animals and humans consume crops that contain high concentrations of the metals or via direct inhalation in a highly polluted area (Silbergeld, 2003), which in this case are predominantly in the 0-20 m zone from the unpaved road, but in general also the lowest the furthest from the unpaved road.

Different plant species can differ in their degree to take up certain metals and influence the deposition of dust depending on their life history and leaf traits (Filipović-Trajković, 2012). The results of the current study show that *P. glauca* had a greater affinity to accumulate Cu and Zn than *R. trichotomum* but *R. trichotomum* accumulated more Cr, Ni and Pb (Fig. 3.4). The reason why some plant species take up more of a specific metal than other plants is that in order to survive plants might require more of a specific metal compared to other plants, even if they occur together in the same community. High concentrations of metal ions can be non-essential or essential, meaning in overabundance a particular metal can be toxic, however depending on the species there are several pathways plants use to deal with excess metal contamination. All plants have basal metal tolerance and some are even capable of hyperaccumulation, however, the general mechanisms used are sequestration processes, exclusion and chelation by moving metals from one organ to another; for example, certain metals might be moved from the leaves to the roots (Viehweger, 2014). Leaves of *P. glauca* and *R. trichotomum* were sampled from plants growing adjacent to one another suggesting that species are able to differentially engage in these processes, which might help explain why different concentrations of the same metal were found within and on their leaves.

Along the roadsides of Sydney, Australia, PM load on the leaves of 16 different species and their important leaf characteristics were determined by Leonard et al. (2016). They found that particulate matter was heavily determined by leaf shape and that PM on lanceolate leaves > PM on obovate leaves > PM on elliptic leaves > PM on needle-like > PM on linear leaves. The leaves with a narrow base tend to flutter more, which increases the chances of removing PM. The plant species with leaf hairs accumulated significantly more PM compared to plant species without leaf hairs. The leaves of *P. glauca* are linear whereas the leaves of *R. trichotomum* are elliptic, which could explain why the concentrations of three out of the five metals in *R. trichotomum* exceeded that of *P. glauca*. Leaves of *P. glauca* are tiny and curved towards the ground, which would increase the chances of dust removal, whereas the leaves of *R. trichotomum* are hairy and waxy that fold inwards, favouring dust pick up and retention. Plant species type explained 47.4% of the variation ($F = 28.8$, $P < 0.001$) in leaf elemental traits (leaf metal and C and N isotope abundance). Distance is a significant explanatory variable but account for only 11.8% ($F = 3.5$, $P < 0.001$) of the variation in leaf elemental traits. The significant influence that species type had on the variation of leaf elemental traits however, indicates that different species will have different concentration of each metal in and on their leaves.

According to Påhlsson (1989) out of all of the five metals, Zn is the least toxic and plant growth will only be affected at 1000 µg kg^{-1}; however, *R. trichotomum* had Zn concentration levels of 8000 µg kg^{-1} and *P. glauca* had concentration levels of 19 805 µg kg^{-1}. These concentrations are however are not what was found within the leaves alone but it is a combination of Zn concentrations deposited dust on the leaves and the concentration within the leaves itself. Thus, it is not sure whether or not Zn levels within plants exceeded that of 1000 µg kg^{-1}, but if deposited dust on leaves gets absorbed by the plants this could pose a potential threat to plants especially within 0–100 m from the road, where Zn concentration was highest. At 100 to 200 µg kg^{-1} for Cu and even lower concentrations for Pb plants may experience metabolic process interference which can result in a decreased growth rate (Påhlsson, 1989). From 0–20 m Cu and Pb accumulation in and on *R. trichotomum* leaves were 3197 µg kg^{-1} and 1102 µg kg^{-1}, and for *P. glauca* it was 14811 µg kg^{-1} and 345 µg kg^{-1}, respectively. Copper poses a potential toxic threat for *R. trichotomum* especially 0–100 m from the road, whereas Pb poses a potential threat for *P. glauca* at especially 0-20 m. Ni can cause a nutrient imbalance in some plant species and impair membrane functions while Cr if in high concentrations, can cause chlorosis in young leaves and root injuries and both of them were highest 0–20 m away from the road (Yadav, 2010). Some plant species, however, are known as hyperaccumulators and grow well of even if concentrations of metals Cu, Cr, Ni, Pb, or Co exceed 1000 µg kg^{-1}. Thus, the focus of management regarding plant toxicity should be given to plants that grow next to the road to approximately 100 m in the field.

In this study I wanted to determine the potential overall metal concentration of common metals in road dust that plants may potentially absorb, and thus it was necessary to determine both the concentrations of metals in and on the plants. Future studies are recommended to do this separately as separate variables will allow different interpretations of results and different relationships to be formed. The ICP analysis has conservative limits since its LOD (lower limit of detection) can be quite high for some metals, which is why it failed to determine the concentrations of one of the metals Hg (lower limit of detection= 82.3 µg kg^{-1}) and as such it was the more conservative option not to separate inner and outer leaf concentrations as concentrations in either might not have exceeded the LOD. Future studies should thus carefully consider which metals they want to incorporate into their study by looking at the type of metals sources within the particular area and the plant species in context so that the metals chosen will be in abundance. It is also recommended that studies in the future should look at other variables such as concentrations of essential minerals and nutrients (phosphorus, nitrogen, and potassium), symbiotic relationships, gas exchange rates, plant leaf age and lifespan all of which can influence the values of $\delta^{13}C$, $\delta^{15}N$ and SLA.

In conclusion, even over a small scale the plant species responded differently to dust deposition from unpaved roads due to the different physical and physiological characteristics each of them possessed. It would thus be optimal for management to not engage in a simple one-way approach, but should be adaptable according to the specific plant species and to a lesser but still significant extent, distance away from unpaved roads. This is particularly important as differences in concentrations of toxic metals on plants can induce selective feeding of herbivores, which may change the structure of plant communities if selective feeding facilitates dominance of only a few species by reducing competition in the plant community. Furthermore, toxic metals pose a great threat to plants near the proximity of the road (0-100 m) but based on the different $\delta^{13}C$, $\delta^{15}N$ and SLA values found, the response of each species was unique indicating a difference in adapting and coping mechanisms plants engaged in, which is based on their specific resource requirements in that time and space. However, what may be of even greater concern are the toxic metals that are transferred along the food webs to the primary and secondary consumers in a community, including humans, but particularly animals that are not as well adapted to cope with metal toxicity (three out of five metals also pose a carcinogenic threat). Thus, based on the results management should try to mitigate the concentrations of all five metals since Cr, Pb and Ni have carcinogenic potential, Zn has the highest concentrations of all the five metals, and Pb can be toxic even in low concentrations. However, before concentrations can be mitigated the sources of each respective metals should be investigated first, in order to establish the most effective control-strategies. The other main concern is food products such as agricultural crops grown in close proximity of unpaved roads and the influence it will have on human well-being and economic losses. As such management should be adaptive to cope with differential physiological responses of plants but also be efficient and focus resources on plants near the vicinity of the road where the influence of dust on leaf elemental traits is greatest, which is especially important in the Karoo where plants are already under pressure to cope with harsh environmental conditions.

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Chapter 4: Microbial diversity in dust generated from unpaved roads in the Karoo

4.1. Introduction

Dust has the potential to significantly alter soil microbial community composition, which has been observed by several research groups (e.g. Hanson et al. 2016 and Luongo et al. 2016). Vehicle-generated dust, especially derived from unpaved or gravel road surfaces, can be transported over great distances (Kuhns et al. 2008). The microbial community of the road dust might potentially introduce new microorganisms into the recipient landscapes, changing the microbial composition of those affected soil areas (Tang et al. 2018). There are, however, little general information available across ecosystems or even scales. For example, at the local scale, questions remain as to the number of microbes that dust typically carries; whether fungi or bacteria are more likely to be transported with dust; and what the potential impacts of dust in the deposited ecosystems will be (USGS, 2013). The scientists of the USGS (United States Geographical Survey) estimated that thousands of microbial species can be carried by 1 g of dust, which may be composed of some animal and plant pathogens and a variety of other microbial types (fungi, bacteria and viruses) that can have a negative or positive impact (depending on the species being transported) in both marine or terrestrial ecosystems (USGS, 2013).

Wet-deposited dust by rain is often understudied, and when Itani and Smith (2016) became aware of aeolian dust widely transporting microorganisms, they tried to evaluate the microbial diversity of deposited dusts in an eastern Mediterranean region. They sequenced 25 fungi and 93 bacteria from deposited samples, and found diverse assemblages of Ascomycota fungi and bacterial phyla. The microbial diversity differed significantly between rain dust samples but even greater differences in microbial species were found between clean rain and dust-containing rain. Similarly, Zhao et al. (2014) found high concentrations of microorganisms in transported dust originating from livestock production. The airborne dust was mainly comprised of Gram-positive bacteria and fungi, yet only consisted of a small proportion of the microorganisms present in the source. Some of the transmitted microorganisms decreased in abundance due to biological and physical decay that depend on the size of the microorganisms and environmental factors, such as temperature, toxic gasses and humidity. Another interesting question, but for which there is scant data, is whether dust characteristics like load and particle size would influence dust microbial composition.

Claub (2015) investigated how the distribution of particle size affects the number and type of microorganisms found in airborne dust. A six-stage Andersen sampler was used to sample particles smaller than 12 µm due to its inefficiency at sampling larger particle sizes. In different environments there was a difference in the median particle size distributions that carried fungi and bacteria. The size of the bacteria-laden particles depended on the mechanism of aerosolization and the particular source. For example, differences in bacterial distributions were found in particles larger than 7.2 µm between dust from livestock husbandry and ambient dust in the air. Most of the particles carrying fungi were between 1 µm and 3.2 µm; however, the particle sizes responsible for carrying moulds depended on the spore size or cell of the predominant species. Even though there is a lack of information on the abundance of microorganisms in particles > 12 µm and especially > 20 µm, many studies suggest that most microorganisms can be found in airborne particles larger than 10 µm. Studies show that aerosols (a suspension of fine particles in liquid gas) contain many bacterial populations and a variety of other microorganisms that impact the environment when deposited (Abed, 2012). The combination of dust and its associated microbes may thus impact ecosystems near roads where vehicle-generated dust might introduce species.

Dust pollution due to human activities can impact microbes in the soil by changing soil characteristics. Amani et al (2018) examined soil samples collected 250, 500, 1000 and 2000 m away from a cement plant. The cement dust induced changes in the soil pH and deposited metals, which affected the physiological processes and composition of bacteria in the soil. In areas closest to the source, the bacterial populations declined compared to distances further away. Similar results were found for fungal populations. Zhalnina et al. (2014) also indicated that changing soil pH was a strong determinant of microbial composition. Similarly, high soil salinity can be very stressful towards soil microbial flora, especially in a dry hot climate when soil humidity is low (Silva and Fay, 2012). Thus, road dust, generated from roads often built from importing sand and other materials, does not only have the potential to affect local niches by introducing new species, thereby altering native microbial diversity, but may also reduce the microbial diversity along hundreds of kilometres if the road materials are depauperate in microbial diversity. Conversely, although less likely, if the road source has a healthy microbial community, then the road dust could fertilize any adjacent, disturbed areas.

Over the last few decades various researchers assessed the impacts of road dust on key soil properties that may indirectly affect plant communities, but most overlooked the transportation of soil microorganisms derived from a road surface. Microorganisms constitute up to <0.5% (w/w) of soil mass and play important roles in ecosystem functioning due to their ability to impact soil processes and properties (Balasubramanian, 2017). The main aim of this Chapter was to determine the effect of dust generated from unpaved roads on microbial dispersion patterns, by comparing the species richness and assemblage composition of fungi and bacteria along increasing distances from the unpaved road. Furthermore, the impact of pH and soil salinity (EC) on the microbial composition and richness pattern will be evaluated along the distance gradient. I predict that the dust generated from unpaved roads would have a similar microbial composition and richness as the road, which should impact the soils along the distance it travels into the matrix of the road. Thus, microbial composition and richness should be similar between the road and distances away from the road and both pH and EC will be significant factors to drive this pattern.

4.2. Materials and methods

4.2.1. Study site and sampling procedure

Soils sampling followed the study sites and distance-transect layout in Chapter 2 and Chapter 3. In other words, within each of the four distance categories (D1 = 0–20 m; D2 = 20–100 m; D3 = 100–400 m; and D4 = 400–1000 m) four soil samples were taken from the surface to a depth of between 5 and 10 cm using a trowel. In addition, four soil samples were also sampled from the road, thus a total of 20 soil samples were sampled at each site. Dust from the MWAC (Modified Wilson and Cooke) samplers (see Chapter 2) was also analysed for microbial species composition and richness. Soil samples from the four distances including the road (Road, D1, D2, D3, and D4) were combined and top and bottom dust samples from four distances (D1, D2, D3, and D4) were combined separately. In total five soil samples and eight dust samples at each site should have been analysed. However, due to the low dust loads captured by the samplers, especially at Wolwekraal (see Chapter 2) several of the combined top/bottom dust samples in a distance category were not analysed, including one top dust sample at SKA and all of the top dust samples and one bottom sample at Wolwekraal (combined dust samples did not weigh up to 0.25 g). More specifically, dust and soil samples analysed at both sites were as follows:

- At Wolwekraal five combined soil samples were analysed, which included one road sample (SoilRoad) and four soil samples at distances 0-20 m (SoilD1), 21-100 m (SoilD2), 100-400 m (SoilD3) and at 401-1000 m (SoilD4). Only three of the four combined bottom dust samples from three distances were analysed including at 0-20 m (D1Bot), 21-100 m (D2Bot) and 401-1000 m (D4Bot). None of the combined top samples were analysed at Wolwekraal.
- At SKA similarly, five combined soil samples were analysed which included one road sample (SoilRoad) and four soil samples at distances; 0-20 m (SoilD1), 21-100 m (SoilD2), 100-400 m (SoilD3) and at 401-1000 m (SoilD4). However, at SKA a total of seven out of eight combined dust samples were analysed including three top samples at three of the four distances; 0-20 m (D1Top), 21-100 m (D2Top) and 101-400 m (D3Top) and four bottom samples at four distances; 0-20 m (D1Bot), 21-100 m (D2Bot), 101-400 m (D3Bot) and 401-1000 m (D4Bot).

4.2.2. Microbial analysis

Within 24 h of sample collection the DNA was extracted from approximately 0.25 g of combined soil and dust samples. A total of 100 µl of genomic DNA extracted per sample was used with the soil. The ZR Soil Microbe DNA kit was used for genomic DNA extraction, and after the extraction process all samples were stored at -20 ºC for subsequent PCR use (Smart, 2018). The 16S rRNA region was amplified by fluorescently labelled primers in order to determine the structure of bacterial and fungal communities by using the ARISA procedure (Automated Ribosomal Intergenic Spacer Analysis) (Brown et al. 2005). This is a fingerprinting technique that does not distinguish at any level of identification (species, families, genus etc.)

4.2.3. Soil pH and EC (electrical conductivity)

After sampling, soils samples (20 samples per site) were air-dried for approximately 45 days and were sieved through a 2-mm mesh. Distilled water was used to disperse soil aggregations in a 1:5 soil water suspension. A total of 20 g of soil was suspended within 100 mL distilled water. The solution was mechanically shaken for one hour at 15 rpm. A pH and conductivity meter were used to determine the respective pH and EC of the soil samples. A mechanical stirrer was used to stir samples between each sample measurement and both meters were standardised against known buffer solutions before measurements took place.

4.2.4. Statistical analysis

To determine the similarity or dissimilarity between soil and dust samples across the distance for both SKA and Wolwekraal, detrended correspondence analyses (DCAs) with an accompanying diversity (species richness) analysis was performed using CANOCO 5 (Microcomputer Power, Ithaca; Ter Braak and Šmilauer 2012). As there was not enough dust sampled within the samplers to conduct pH and EC measurements, these variables were only measured for soil samples at both sites, and a redundancy analysis (RDA) was done to determine the association between soil pH and EC on soil bacterial and fungal OTUs. Finally, since dust load and particle size had been determined for dust caught by the samplers (Chapter 2), their association with bacterial and fungal species richness and community composition could also be determined. However, the impact of dust load and particle size on dust samplers were only determined at SKA since not all samplers were analysed for microbial diversity at Wolwekraal due to dust volume limitations. For this analysis another DCA was performed. All variables were standardized.

4.3. Results

4.3.1. Microbial species richness

Bacterial and fungal species richness for soil samples and dust samplers were determined at both sites. At SKA, total bacterial species richness (bacterial OTUs = 176) was marginally less than total fungal species richness (fungal OTUs = 179) in the dust samples, and total bacterial species richness (bacterial OTUs = 71) was more than total fungal species richness (fungal OTUs = 47) in soil samples. At Wolwekraal, total bacterial species richness (bacterial OTUs =

108) was higher than total fungal species richness (fungal OTUs = 71) in the dust samples, and so too total bacterial species richness (bacterial OTUs = 165) was more than total fungal species richness (fungal OTUs = 113) in soil samples.

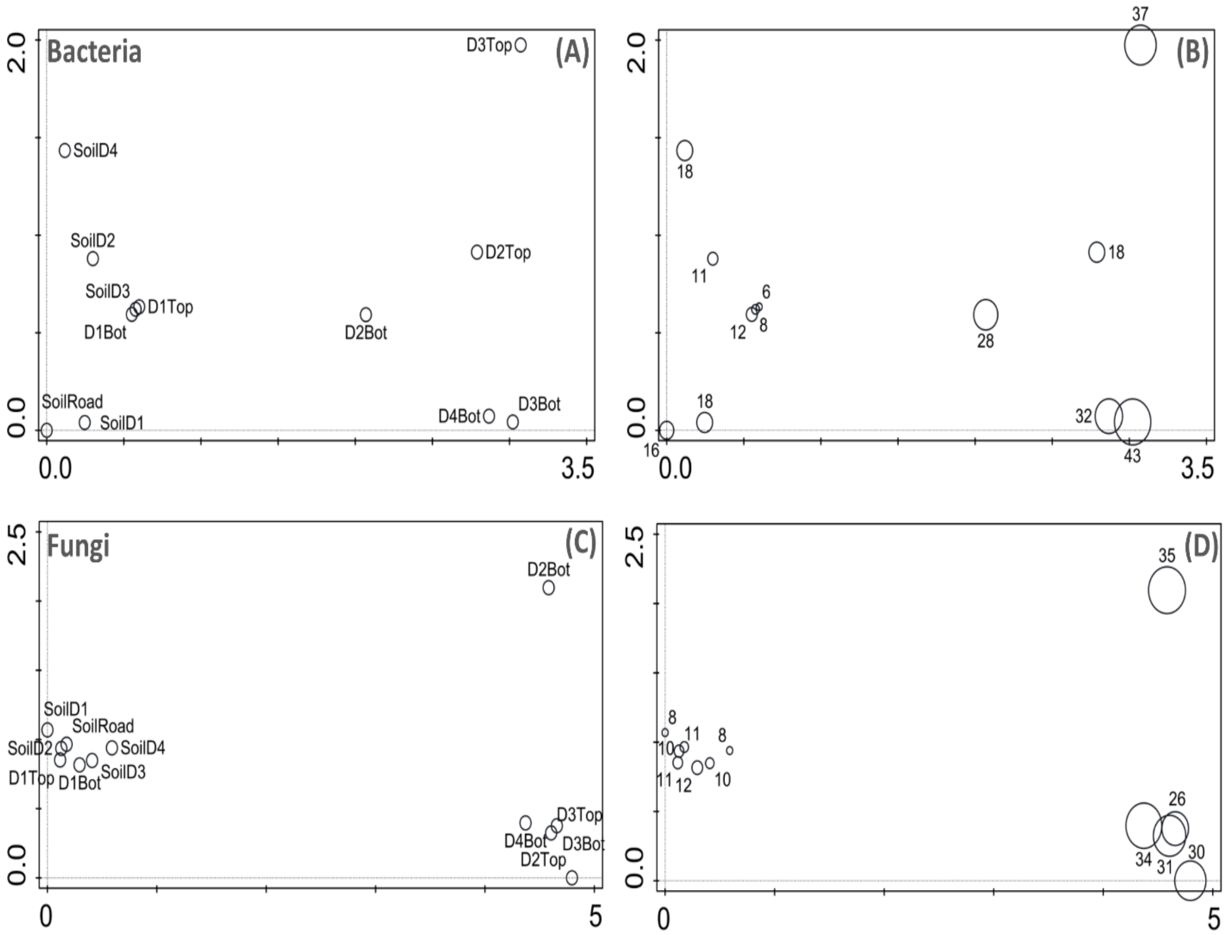

Figure 4.1. Detrended correspondence analyses (DCA) showing bacterial composition dissimilarity (A) and species richness (B) between soil samples and dust samplers at SKA, as well as the fungal composition dissimilarity (C) and species richness (D) between soil samples and dust samplers at SKA. Soil = Soil samples; Top/Bot = Top or bottom dust samples; D1 = 0–20 m, D2 = 21–100 m, D3 = 101–400 m and D4 = 401–1000 m.

At SKA, for both bacteria and fungi, the clumped soil samples included the two dust samplers nearest to the road (D1Top and D1Bot), which had lower species richness (bacterial OTUs = 89 and fungal OTUs = 70) compared to the other group where the remaining dust samplers clumped (D2Top, D2Bot, D3Topt, D3Bot and D4Bot; bacterial OTUs = 158 and fungal OTUs = 156) (see Fig. 4.1). Thus, soil samples taken to a depth of approximately 10 cm here had significantly less microbial diversity than what was caught in the samplers beyond 20 m from the road. It, therefore, appears that the nearest sampler has caught the microbes from the looser road soil with the farther samplers accumulating microbes from a different source.

At Wolwekraal, bacterial species richness was the lowest at the road (SoilRoad OTUs = 13) and the nearest dust sample (D1Bot OTUs = 31). Similarly, the two samples that had the lowest fungal species richness was the road (SoilRoad OTUs = 5) and the closest soil sample (SoilD1 OTUs = 10) (Fig. 4.2). It therefore also appears that, at least for fungi dispersion, the road having fewer species influences dust microbial diversity in the 0–20 m road verge.

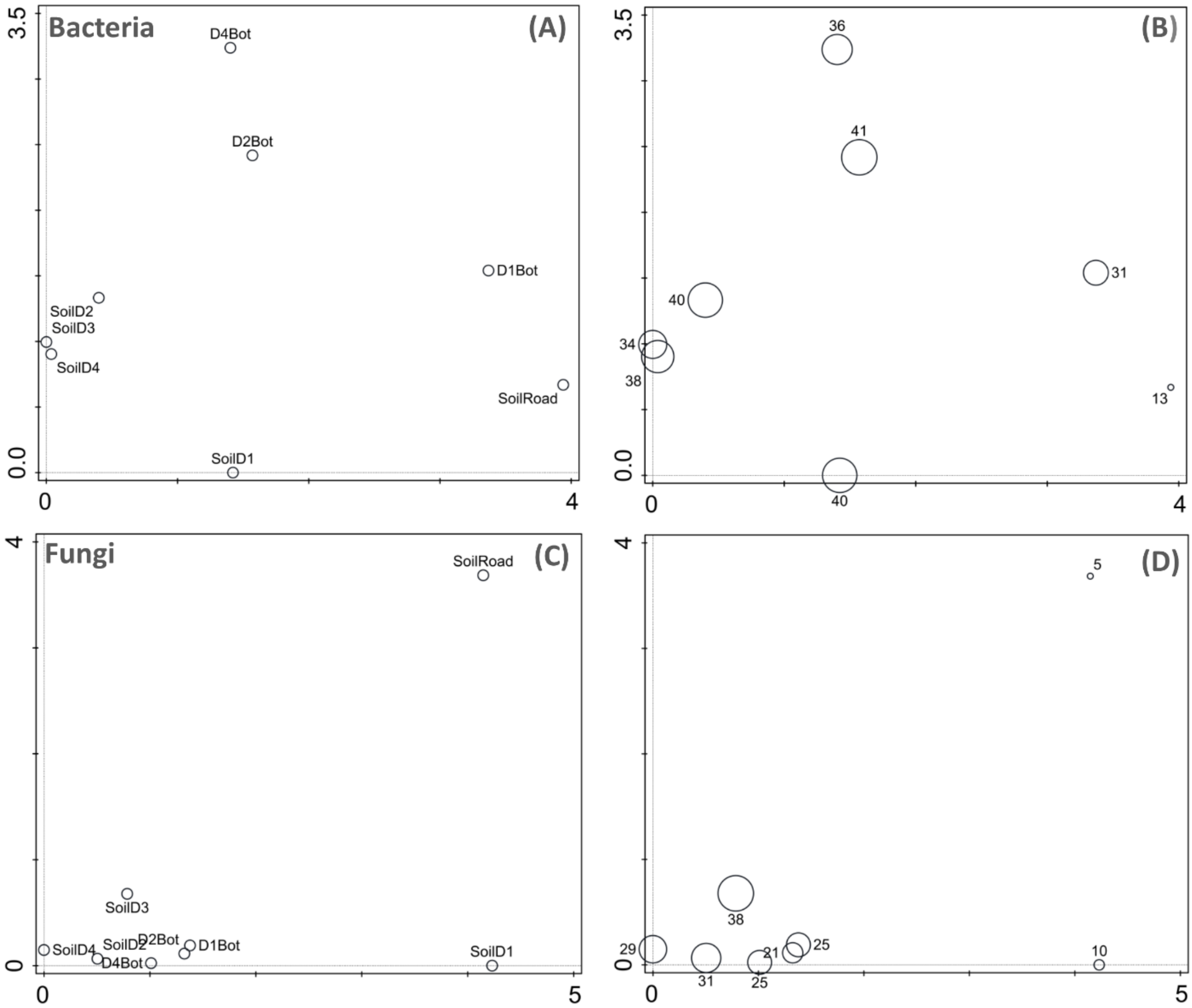

Figure 4.2. Detrended correspondence analyses (DCA) showing bacterial composition dissimilarity (A) and species richness (B) between soil samples and dust samplers at Wolwekraal, as well as the fungal composition dissimilarity (C) and species richness (D) between soil samples and dust samplers at Wolwekraal. Soil = Soil samples; Top/Bot = Top or bottom dust samples; D1 = 0–20 m, D2 = 21–100 m, D3 = 101–400 m and D4 = 401–1000 m.

4.3.2. Microbial composition of dust in the samplers and soil

Both bacteria and fungi display similar patterns in terms of species composition at both sites (Figs. 4.1 and 4.2). At SKA, there was a clear difference in bacterial species composition between dust samples and soil samples, with the only exception being the species composition of the dust samples closest to the road (D1Top and D1Bot) grouping strongly with the soil samples compared to the other dust samples at distances further away from the road (Fig. 4.1). A similar pattern for the fungal species composition was found at SKA. In fact, the difference between the composition of the dust samples and soil samples was even more profound for fungi (closer groupings using Bray-Curtis distances, with the first DCA axis being > 4) (Fig. 4.1)

At Wolwekraal, bacterial composition followed a similar pattern as for SKA, where the soil from the road (SoilRoad) and from the bottom sampler at the distance closest to the road (D1Bot) had a similar assemblage. These two samples were most dissimilar from the soil and dust samples, in terms of bacterial species composition (Fig. 4.2). However, for fungi, the road soil sample was more different than any other sample taken, most likely due to the low number of OTUs found ($n = 5$). In turn, the D1 soil sample had the second lowest species richness and its fungal community appeared to be closer to the road soil sample than the rest (Axis 2 is shorter than Axis 1, explaining less of the variation). In general, dust samples overlapped more with soil samples for fungal species composition, with only species richness tending to increase away from the road (Fig. 4.2).

4.3.3. Correlates of soil and dust microbial species composition and richness

At both sites, fungal and bacterial species composition correlated with EC and pH (Fig. 4.3). This pattern was mainly driven by the high EC and lower pH of the unpaved roads. These species-depauperate roads are thus edaphically different from the matrix soils, with only the soil microbial communities closest to the road also showing a dissimilar EC and pH association compared to the soils further in-field.

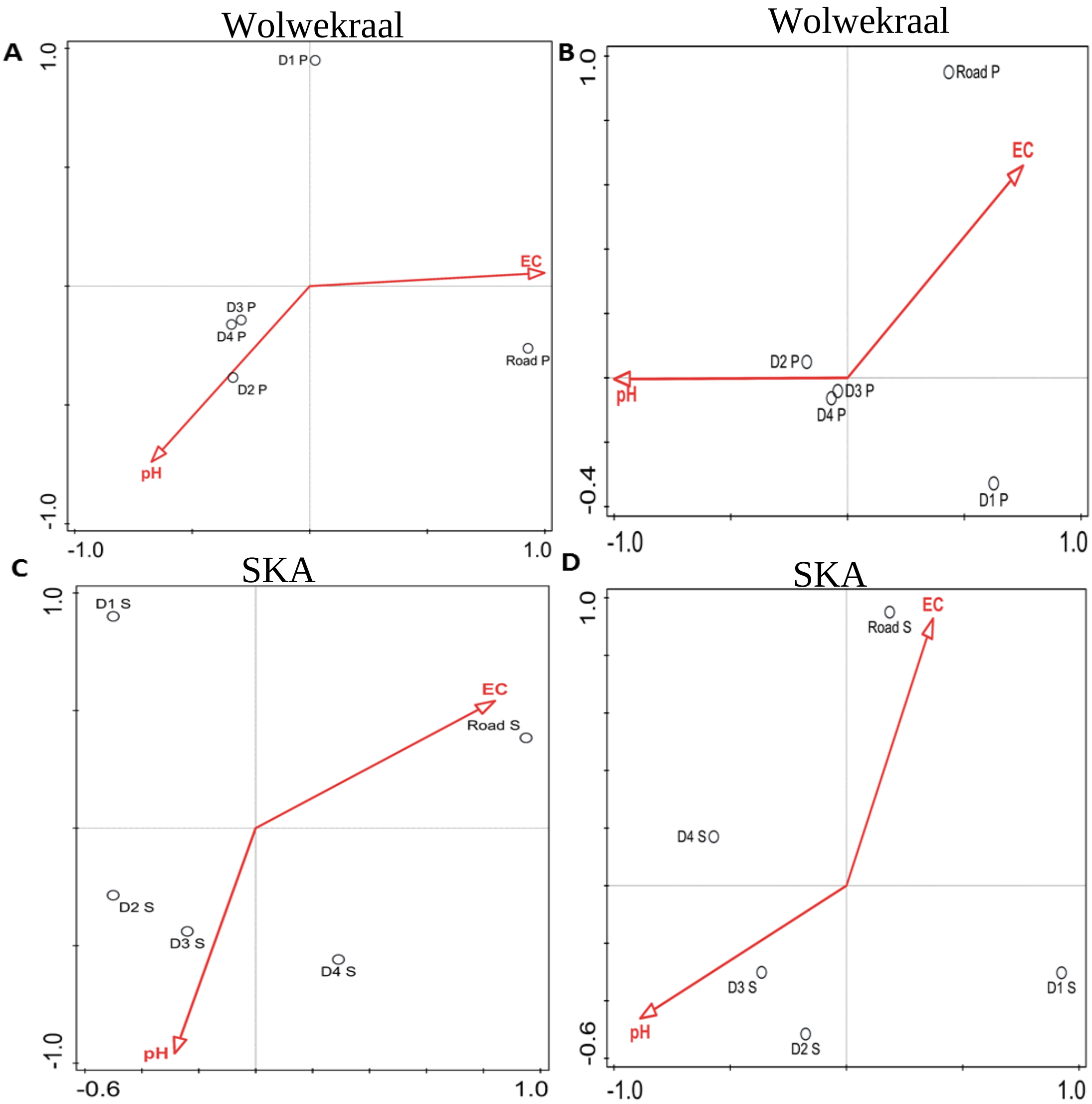

Figure 4.3. Redundancy analysis (RDA) depicting the association between soil electrical conductivity (EC) and pH on (A) bacterial community composition of soil samples at Wolwekraal; (B) fungal community composition of soil samples at Wolwekraal; (C) bacterial community composition of soil samples at SKA; and (D) fungal community composition of soil samples at SKA. Soil = Soil samples; D1 = 0–20 m, D2 = 21–100 m, D3 = 101–400 m and D4 = 401–1000 m.

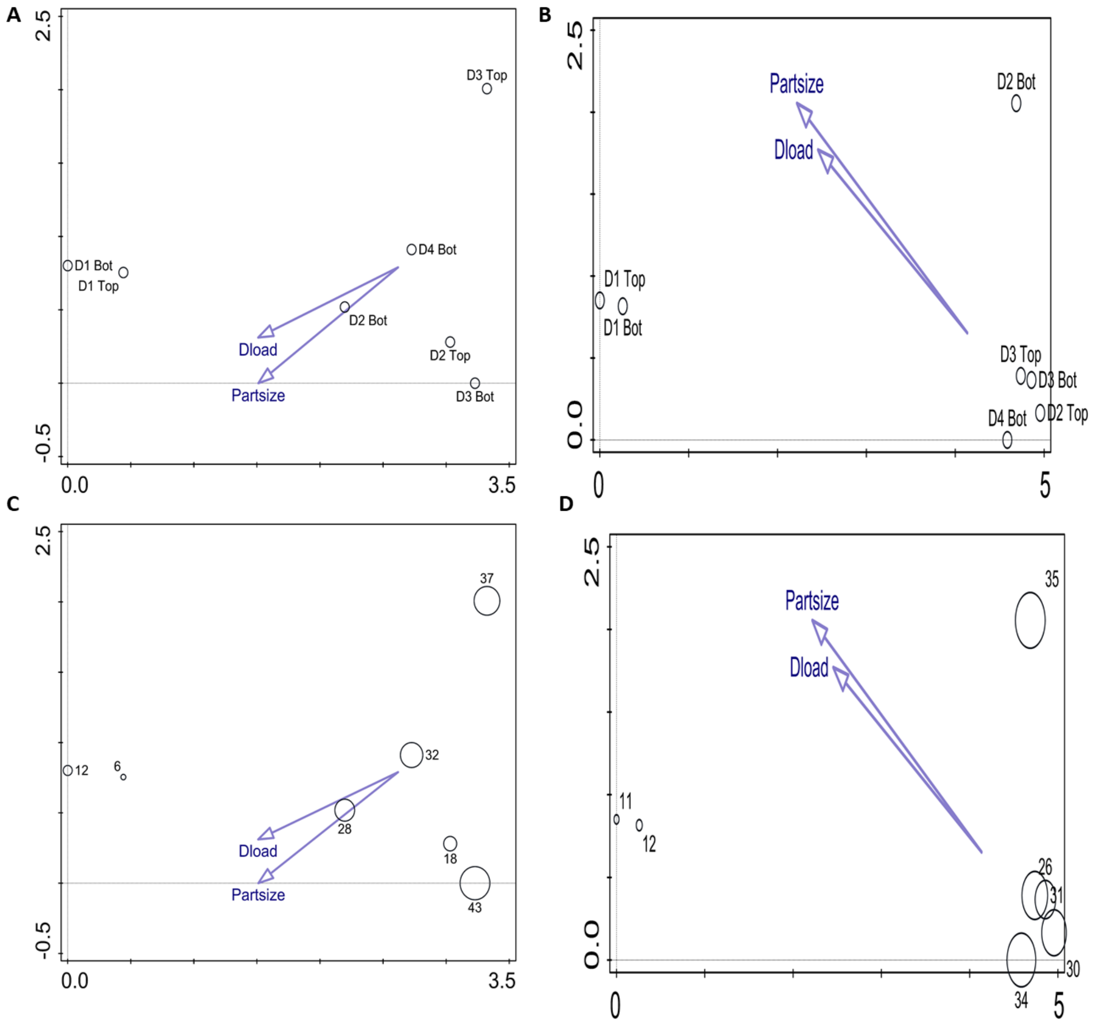

Figure 4.4. DCAs showing the association of dust load and particle size on (A) bacterial species composition measured within dust samplers; (B) fungal species composition of dust samplers; (C) bacterial species richness of dust samplers; and (D) fungal species richness of dust samplers. Samples from SKA only.

Dust load and particle size clearly associated with the different bacterial and fungal communities, with a more positive correlation next to the road (D1Top and D1Bot; Fig. 4.4). The same trend was true for species richness (Fig. 4.4). Higher dust loads, as expected directly next to roads (see Chapter 2), are thus also associated with lower fungal and bacterial diversity.

4.4. Discussion

At SKA, the bacterial and fungal communities in soils from the unpaved road overlapped with the bacterial and fungal composition from all the soil samples taken, regardless of distance, but interestingly also with the communities found in the dust of the samplers closest to the road (0–20 m). In contrary to expectations, the bacterial and fungal composition of the dust samplers from D2 to D4 (21–1000 m) were distinct from the abovementioned samples. Thus, the road at SKA does influence the dust microbe communities close to the road in samplers and is similar to soils in the 5-10 cm depth. Further in-field, however, another source of microbial diversity increased dust diversity in samplers, but not soil diversity. Dust samplers closest to the road should have the greatest probability to capture microbes coming from the road compared to the samplers at greater distances away from the road. In turn, the Wolwekraal road had the lowest species richness of all for both fungi and bacteria, and this drop in diversity relative to other samples was also seen in the soil and sampler closest to the road, although varying between fungi and bacteria. The prediction that the generated dust should be more similar in community composition than the road soil it originated from is thus only partially true, and particularly only clear in the 0–20 m road matrix where the highest dust loads and particle sizes are expected. No study before this current study tried to evaluate microbial composition along a distance gradient from an unpaved road, especially by using dust samplers as the main dust capturing method; however, there are a few studies that recorded microbes being transported to other areas within dust (e.g. Roselli et al. 2015; Itani and Smith, 2016; Zhao et al. 2014).

Not only does dust transport nutrients but it may also convey viable microorganisms consisting of different species. This may be a concern as deposited inflowing viruses, fungi and bacteria may potentially harm ecosystem health (Roselli et al. 2015). In this particular study changes in microbial composition over very short distances were observed whereas in most literature global transportation of microorganisms in dust is usually determined, and whether over long or short distances an increase in diversity and changes in microbial composition were observed in the deposited regions (Roselli et al. 2015; Prospero et al. 2004; Hanson et al. 2016; Itani and Smith, 2016. An example of short distance transportation of microbes in dust includes a study conducted by Gardner et al. (2012) that determined the bacterial species composition from three agricultural fields varying in soil characteristics, organic matter and management history using a portable wind tunnel 25 to 50 m away from the sources. They found high bacterial diversity (approximately 3000 sequences) in transported dust that differed in composition between coarse and fine particles due to the different substrate that different particle size provides in terms of organic matter and mineral content. There was a decrease in bacterial diversity at the soil source; some of which species that disappeared have important ecological roles in nutrient cycling and enzyme activity in the soils of the farms, important aspects of agricultural productivity (Gardner et al. 2012). Transportation of microorganisms and its impacts is thus not always negative in the deposited areas, because although there was a loss of important bacterial diversity from the farms in the study of Gardner et al. (2012), other ecological niches may benefit from these important microorganisms, increasing their soil diversity Thus, depending on the microbial species within the road soil, which depends on the road soil characteristics, the soil productivity of dust receiving areas depend on whether favourable or unfavourable microorganisms were transported.

In the case of the current study dust transported from the road had low species richness and influenced the samplers in the 0-20 m zone and associated soil samples by diminishing their microbial richness and diversity as well. Depending on which species road dust impacted in the soil, especially if microbes play important ecological roles in nutrient cycling and soil stability, a negative effect on soil productivity may be found that could indirectly decrease plant fitness. At SKA all of the soil samples, the road and the samplers (D1Top and D1Bot) at 0-20 m from the road had lower bacterial and fungal species richness compared to the dust samplers at distances further away from the road. Similarly, although from a different source, Amani et al. (2018) conducted a research study to determine the impacts of microbial cement dust on fungi and bacteria 250, 500, 1000 and 2000 m in the soil away from a cement manufacturing plant. There was a decrease in bacterial and fungal populations closer to the cement industry. The cement dust induced changes in soil chemistry, which resulted in an overall decrease in soil microbial diversity.

Thus, based on the results soil pH, soil EC, dust load and particle size, along with other important soil and dust properties that were transported from unpaved roads, played a role in this pattern observed in samplers at 0-20 m and soil samples further in-field. Another possibility that might have led to this result is that the topsoil at all the distances and the samplers closest to the road may have received pathogenic species from the road, which could have limited species richness at those points in space and time. The other samplers away from the road had a lower probability of receiving dust from the road, but higher species richness in those samplers may indicate that microorganisms were aerially captured from other source areas. This is only an assumption since species within samples were not identified and as such there is no evidence that pathogens were received from the road dust. An example of pathogenic transportation includes a study conducted by Shin et al. (2000) that identified many fungal spores and bacteria in dusts transported over many years from Africa to the Caribbean coral reefs. Within these dust *Aspergillus sydowii* was found, which were linked to coral reef decline. Finally, the influence of soil depth, changing microbial diversity should not be underestimated. At SKA the topsoil structure was loose and could easily be eroded by wind exposing the deeper soil that was sampled. Fierer et al. (2003) showed that microbial diversity and biomass could decrease even from a depth of 0-5 cm to 5- 15 cm. At Wolwekraal, however, the topsoil was covered by biological crusts that are more difficult to erode. Steven et al. (2013) demonstrated that biocrusts and the soil just below the crusts are similar in diversity. It was likely that the less diverse deeper soils at SKA and the more diverse biological crusts at Wolwekraal, were sampled, displaying the different species richness patterns observed between the two sites.

An interesting finding of this study was that for both bacteria and fungi the species composition of the road at Wolwekraal differed markedly from the species composition of all of the soil samples. One would expect that since the soil in the Wolwekraal Reserve received dust from the road, that all of the soil samples and road samples would have similar microbial compositions, however the opposite was observed. One particular cause for this observed pattern might be due to the fact that Wolwekraal often brings in soil additions from other areas to improve the soil structure of the road. These soil additions may have had other microorganisms that were novel to the particular site.

In this particular study, soil pH and EC were associated with both bacterial and fungal species composition between soil samples from the road and soil samples at distances away from the road. Certain microbial species are more associated with saline soil conditions. Pecher et al. (2019) found that when salting was used to de-ice roadways it led to an increase in soil salinity levels. The microbial communities that were exposed to road salt runoff increased in archaea and bacteria halophiles which could have great implications for ecosystem functioning. Soil salinity could thus decrease microbial evenness by favouring the dominance of specific microbial species adapted to saline soil conditions decreasing the overall diversity. Similarly, in a boreal forest, Yan et al. (2017) found that during different seasons and with different nitrogen additions different species dominated the community and these taxa could be used as important biomarkers to how the microbial community responds to these changes.

pH is another important variable that affects soil microbial composition at various geographical scales. Beneficial microorganisms thrive in a pH range between 6 and 7 and soil acidification leads to a shift in which species occur in soils, and their activities are often altered.

This could result in a decrease in the availability of essential nutrients to plants, because immobilization of basic nutrients occurs when there are significant changes in the rate decomposition. Also, pH changes could result in a shift between bacteria or fungi dominated soil since the more acidic the soil the greater the chance to shift the balance from bacteria to a more fungi-dominated soil (Sullivan et al. 2017). This could explain why species richness of bacteria was more than species richness of fungi, since soil pH found in the current study were relatively neutral to alkaline. A study conducted by Wu et al. (2017) found that soil pH was the main determinant of bacterial community structure. Bacterial diversity was much lower in acidic samples compared to neutral samples and along the pH gradient the dominant phyla's relative abundance varied.

At SKA it seemed that dust load and particle size had a strong influence on bacterial and fungal species composition. Bacterial and fungal species richness from the dust samplers closest to the road (D1Top and D1Bot) were also suppressed, possibly due to an increase in particle size and dust load. Claub (2015) and Gardner et al. (2012) had shown that the distribution of particle sizes can affect the number and type of microorganisms found in airborne dust.

Future studies are recommended to identify species within soil samples and dust samplers in order to obtain information regarding the presence or absence of pathogens or beneficial microorganisms, which may aid in the explanation of certain trends that were found. Based on the results of the current study and other similar studies, the impacts of dust on diversity and richness, whether positive or negative, depend significantly on the soil or dust properties from the source. Another important factor to consider is the sampling method implemented in the study, because sampling depth will influence microbial composition and diversity. The soil samples and dust samplers should be evaluated for other important indicators, including those variables not determined within this study, such as soil aeration, soil organic matter content, soil temperature, relative humidity and soil water content (SWC) all which can influence the type of microorganisms found and their relative abundance. In chapter 3, we found high concentrations of metals closest to the road which could have an impact on soil and dust chemistry, in particular, the pH of the samples. The information obtained from all different variables should be used in combination to investigate how it will impact plant fitness in the community.

4.5. References

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Chapter 5: Synthesis

5.1. Site factors introduce differential impacts of road dust and dust generation mitigation approaches

In other similar studies conducted, some researchers recorded higher mean dust loads whereas others found lower amounts than what was found at both sites in the current study (Walker and Everett, 1987; Kaler et al. 2016; Korte et al. 2017). However, it is difficult to compare dust loads among these studies, since they all differed in their dust sampling techniques and research environments. Dust load and particle size displayed different patterns at the two sites, and Wolwekraal did not show the pattern expected, namely consistently decreasing mean dust load and particle size along with distance from the road. Usually, with distance from a road, these two factors consistently decreased and are the greatest nearest the road (e.g., Walker and Everett, 1987; Avon et al. 2013; Kaler et al. 2016). However, the different patterns found at Wolwekraal and SKA indicate that there are underlying site-specific factors (i.e., topography, vegetation height, etc.) that need to be more thoroughly investigated so that more generic trends and relationships may be derived. The results from Chapter 2, Chapter 3, and Chapter 4 suggest that road dust can have significant impacts on plant leaf functional traits and meaningful impacts in altering dust microbial diversity, but again impacts may be site-specific. Where significant impacts may ensue, it would be imperative for conservationists to engage in the concept of triage and focus on mitigation regulations in order to reduce dust generation from unpaved roads before focusing on impacted vegetation areas, to ensure more efficient use of resources pertaining to dust control.

For example, even though dust control methods might differ in cost and practicality, one of the more effective management strategies is reducing vehicle speeds, because vehicle speeds in some situations are proportional to the amounts of dust generated from unpaved roads (Jones, 2000). The number of vehicles that enter an area, public or privately owned, which contain unpaved roads, might be a further factor that might also aid in the mitigation of dust generation. Further, several studies indicated that heavy traffic increases dust loads and releases higher concentrations of toxic metals (e.g., Tanee and Albert, 2013; Nepali and Gyawali, 2001). Other more short-term options include the use of chemical palliatives, waste oils, and water and wetting agents, but before any of these methods are implemented, their potential impacts on the environment should be considered. However, based on the significant findings in regards to chemical (metal concentrations) and microbial impacts of dust, I urge that landscape planners should consider a long-term option such as to permanently seal unpaved roads, if none of the dust control strategies mentioned above are effective at mitigating dust generation.

Because there was a difference found in dust particle size between the surface (coarser) and 1.3 m above the ground (finer), it is recommended that managers engaged in cultivating or conserving tall (e.g., tree crops) or short plants (annual crops) are engaged differentially. However, the sampling procedure, i.e., dust trap choice, in future studies should be carefully considered and linked towards the objectives of their research study, because dust traps differ in their sampling effectiveness in certain situations for e.g. the MWAC (Modified-Wilson and Cooke sampler) and VS (Vaseline sampler) differ in their effectiveness at sampling different sizes of dust particles and at different heights (Youssef et al. 2008).

5.2. The impact of road dust on leaf traits (leaf metal and C and N isotope abundance)

Studies regarding heavy metal or trace metal deposition concentration from a road differed in terms of which metals dominated (e.g., Jardat and Momani, 1999; Tanee and Albert, 2013). This may be the result of the interaction between different variables in and surrounding the research area. The traffic type and volume, the plant species, and the differences in leaf characteristics that either mitigate or accumulate more metals, and the source type and amount are all crucial factors that need more focus when similar studies are conducted. The dust-borne metals that I investigated in this study all behaved in a different way as some metals displayed a clear pattern of consistently decreasing concentrations along a distance gradient, while others did not display this pattern. Some of the metals had the highest concentrations along the edge of the road (0-20 m), but others did not as their concentrations were highest at distances further away from the road. This is an important finding as some of the metals (Cr, Pb, and Ni) are carcinogenic and pose a threat to the health of humans, agricultural crops and livestock, or any animals that feed on the crops. As such, the same management strategies should not be directed at pooling the metals together, but it is recommended that they are managed differently according to their specific behaviour.

Plants that are closest to the road receive the highest dust load and also very high metal concentrations; however, it doesn’t seem to significantly hamper the WUE, nitrogen uptake, or SLA ability of any of the four species. This could be the result of the evolution of mechanisms by plants to adapt to the impact of dust on roadsides over many years. For example, Saneoka and Ogata (1987) found that plants were most under stress due to the intensive deposition of dust close to the source and therefore adapted by synthesizing more wax on their leaves to decrease water evaporation and be more drought resistant. Distance was a significant factor that influenced variation in leaf elemental traits (leaf metal and C and N isotope abundance) at SKA, but even more significant was plant species. Plant species have different limitations and adaptation to dust loads, particle size, and heavy/trace metals, and as such, they responded physiologically differently. Based on the results presented, SLA cannot be used as a predictor of these physiological traits ($\delta^{13}C$ and $\delta^{15}N$), but there are many other variables not determined in the current study, which, according to previous research, that may play a significant role in influencing SLA.

5.3. The differential influence of road dust on soil microbial diversity and composition between the two sites

Many studies focused on the physical, chemical, and physiological aspects of road dust and their impacts; however, based on the findings of Chapter 4, I suggest that researchers do further work regarding the microbial aspects of dust and its related impacts on soil and plants. Most literature regarding transportation of microorganism in dust recorded higher diversity in deposition zones (e.g., Roselli et al. 2015; Itani and Smith, 2016), however in the current study I provide evidence that receiving dust from the road at SKA particularly, supresses microbial species richness of the soil between 5 and 10 cm and even in dust samples closest to the road (0-20 m). This could have significant consequences in terms of degrading important ecosystem services that microorganisms provide, such as recycling, increasing the availability of soil nutrients, and stabilizing soil structure. In this particular study, factors such as soil pH, soil salinity levels, dust load, and particle size can all be strong determinants of microbial composition and species richness, but there are several other variables such as soil porosity and humidity that need to be investigated. This is an indication of the strong influence unpaved roads can have at the edges since the pH and EC between the road and distance closest to the road (0-20 m) were most similar compared to distances away from the road. Other possible explanations for decreasing species richness are receipt of pathogenic species and the sampling method (depth of sampling), which also depends on the soil characteristics of the sampling site (biological crusts versus loose soil structure).

Future studies should put more emphasis on identifying which microbial species are transported along the distance gradient. At Wolwekraal the microbial composition of the road was entirely different from the microbial composition of the other soil samples. Soil incoming from other areas could change some of the important soil properties and bring in non-indigenous microorganisms that impact local microbial composition, which in turn may impact plants in the local community. Incoming soil at Wolwekraal could also explain why results found between the two sites were different because, in contrast at SKA, local road dust material was sampled, suggesting that the two road soil surfaces were different (with differential impacts). This is reflected by the different dust physical and microbial characteristics found between the sites. Thus, the impacts of incoming soil from other areas should not be taken lightly as it may induce changes in the soil properties of the road, which may pose a potential threat to the local vegetation community. It is recommended that additions brought in, for example, to improve the soil structure should be carefully considered and should not change the natural local soil chemical, physical, and microbial properties.

5.4. Potential impacts of road dust on grazing behaviour, which could alter local vegetation composition.

Most similar studies evaluated how road dust along with the physical and chemical properties that dust carries impacts plants, but what is seldom studied is how dust deposition on plants would influence the behaviour of primary consumers. Researchers often explain that the taller height of roadside vegetation compared to interior vegetation is due to the more compact soil on the road, causing water along with other essential nutrients to run off the road surfaces to the edges where it is more available to roadside plants. This could explain why roadside plants are usually taller. However, the question remains as to whether higher dust loads and higher concentrations of certain metals could make plants at edges of roads less palatable or even toxic, and as such primary consumers would avoid browsing or grazing such. This could also contribute to taller plant heights compared to the more palatable plants at distances further away from the road, where the chance of primary consumer grazing and browsing increases. Also, selective feeding due to the unpalatability of species covered in dust may induce vegetation structural changes if it leads to the dominance of these unpalatable plant species, which based on the findings in Chapter 2 are shorter Karoo vegetation. This could lead to a shift in Karoo vegetation structure if lower dust deposition on taller plants makes these plants more prone to grazing or browsing. Although O’Farrell and Milton (2004) found that grazing was not the main determinant of plant community composition in road verges within the Karoo (Prince Albert District), Hoffman and Cowling (1990) demonstrated that grazing is still an important factor that cannot be ignored when considering Karoo vegetation compositional changes. Nonetheless, conservation management could, for example, implement buffer areas near roads in order to fence out crop production of grazing areas, to protect both crops from being grazed and animals from consuming heavy metals in and on foliage.

5.5. Future considerations and focus areas regarding road dust impacts

As indicated by a few studies (e.g., Waser et al. 2017; Mandal et al. 2016; Naik et al. 2006), road dust also has the ability to affect autecological factors such as pollination, seed dispersal, and fruiting and flowering and this could have significant social and economic impacts. Thus, an interrelated understanding of different aspects is necessary, and the focus of research should not only be on the ecological effects of road dust. The combination of dust load, particle size, heavy and trace metal concentrations, and microbial composition and richness varied between distances over such a short space, and vegetation may experience differential impacts over this short space. It would thus help that other similar road dust studies would look at a greater variety of factors and study areas and at different scales as this would provide a stronger basis for conservation management and environmental impact assessment to make better planning and management decisions. The main message that needs to be conveyed is that management should implement strategies that focus on mitigating and controlling road dust generation in order to prevent or reduce the potential physical (load and particle size), chemical (heavy/trace metals), microbial and physiological ($\delta^{13}C$, $\delta^{15}N$, and SLA) impacts on plants.

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